RESTRICTED DISTRIBUTION

Accession No. 80-259

Copy No. 9

Issued To R. Carpenter

APPROVAL BY:

MANAGER BIOCHEMICAL RESEARCH

PHILIP MORRIS U.S.A.

RESEARCH CENTER

CHARGE NO. & TITLE: 6906--BIOLOGICAL EFFECTS OF SMOKE

TYPE REPORT: ○ ANNUAL ○ SEMIANNUAL ○ COMPLETION ✓ SPECIAL

DATE: Oct. 21, 1980 PERIOD COVERED:

REPORT TITLE: L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA POINT MUTATION ASSAY--STANDARD OPERATING PROCEDURE WITH SPECIAL EMPHASIS ON THE STATISTICAL ANALYSIS OF THE POSITIVE CONTROL DATA AND MINIMAL CRITERIA FOR AN ADEQUATE TEST

WRITTEN BY R.W. McCuen Rose M. Meyers J.M. Penn

R.W.MCCUEN R.M.MEYERS J.M.PENN

SUPERVISED BY R.A. Pages

R.A.PAGES

APPROVED BY W.F. Kuhn

W.F.KUHN

DISTRIBUTION:

* DR. R. B. SELIGMAN
DR. T. S. OSDENE
* DR. W. A. FARONE
* DR. W. F. GANNON
* MR. L. F. MEYER
* MR. R. N. THOMSON
* DR. R. FAGAN
MR. W. F. KUHN
MR. R. D. CARPENTER
MR. J. L. CHARLES
DR. R. N. FERGUSON
DR. R. A. PAGES
DR. R. W. MCCUEN
DR. R. M. MEYERS
MR. J. M. PENN
MS. D. J. AYERS
CENTRAL FILES (TWO)

*Appendices not included; see memo for explanation.

1003117990

KEYWORDS: Bioactivity, Forward Mutation Assay, L5178Y Cells, Whole Smoke Condensate, Metabolic Activation, Positive Controls, Negative Controls, Data Analysis, Adequate Test, Analysis of Variance (ANOVA), Thymidine Kinase (TK).

SUMMARY

This report presents in great detail how the L5178Y/TK$^{+/-}$ mouse lymphoma point mutation assay is conducted in our laboratory. The initial one-third of this report is referred to as the Standard Operating Procedure (SOP) for this system. The next section of the report concerns the analyses of the control data from more than 2.5 years of work. The main objective is to control some of the variation observed in this assay. From the data analyses, hypotheses were developed and are presented in the final one-third of this report. These hypotheses concern minimal criteria for conducting the test, criteria for accepting an experiment, and criteria for evaluating test results. Major conclusions are made about this assay as it is conducted at Philip Morris.

TABLE OF CONTENTS

TABLE OF CONTENTS

(continued)

INTRODUCTION

The cell line was originally isolated by Dr. Lloyd W. Law in the Laboratory of Cell Biology at the National Cancer Institute in the early 1950's by painting female DBA/2 mice with 3-methylcholanthrene.[55] He isolated several leukemias (among them L1210 and L5178) which were carried as intraperitoneal ascites. [The "L" allegedly stands for Law.]

In 1958, Dr. G. Fischer at Yale University was successful in getting the L5178 cells to grow in culture *(in vitro)* using a semidefined medium which today bears his name.[55] One clone of L5178 cells which he and his colleagues used for much of their work[24,25] was termed L5178Y (the "Y" stands for Yale).

At this point, the cell line possessed many characteristics which made it an excellent choice for biochemical and genetic studies. Some of these were its adaptability, rapid growth in suspension culture (generation time of 10-12 hours), and its stable, near diploid chromosome number (40±1). Because of these characteristics, many different investigations were done with L5178Y cells. In the early 1970's, Clive and co-workers developed and validated the L5178Y $TK^{+/-}$ forward mutation assay which we use at Philip Morris.[5-20,57] This system has been used by many laboratories throughout the world in a battery of tests designed to show the potential *in vivo* activity of pure chemicals and complex mixtures.[1,27,30,32,41] Recently this bioassay has been included in several programs designed to evaluate short-term tests for potential *in vivo* activity.[50,53,54]

The objectives of this report are:

1. to accurately define cell handling and the conduct of testing at PM;
2. to describe the statistical analyses of the positive control data;
3. to define minimal acceptable criteria for testing samples in this assay.

MATERIALS AND METHODS[36]

Abbreviations Used in This Study

2-AAF(2-acetylaminofluorene); ANOVA (analysis of variance); B(a)P [benzo(a)pyrene]; BUdR(5-bromo-2'-deoxyuridine); $BUdR^{Y}$(BUdR-resistant); DMSO (dimethyl sulfoxide); ECT (Elmenhorst cold trapped); EMS (ethyl methanesulfonate); IT (impaction trapped); IMF (induced mutation frequency); S9 (microsomal preparation); MF or MUTFRQ (mutation frequency); $\log_e$ MF (natural logarithm of the MF); NADP (nicotinamide adenine dinucleotide phosphate); % CLNEF (percent cloning efficiency); % SURVI (% survival); SUSPGR (suspension growth); TK (thymidine kinase enzyme); $TK^{-/-}$ (homozygous TK deficient phenotype); $TK^{+/+}$ (homozygous TK proficient phenotype); $TK^{+/-}$ (heterozygous TK proficient phenotype); TFT (trifluorothymidine); TFT^{Y} (TFT-resistant); WSC (whole smoke condensate).

Source of Cells

The thymidine kinase, heterozygotic, clonal line, $TK^{+/-}$-3.7.2 derived from Fischer's L5178Y mouse lymphoma cells[57] was used throughout these studies. Frozen ampoules of these cells were kindly supplied by Dr. D. Clive, Burroughs Wellcome Co., Research Triangle Park, NC.

Chemicals and Media Preparation

The cells were routinely grown in Fischer's Medium for Leukemic Cells of Mice (Grand Island Biological Co. or GIBCO, Grand Island, New York) containing 10% horse serum (GIBCO), Pluronic® solution (1 mg/ml, final concentration of F-68; BASF Wyandotte Corporation, Wyandotte, Michigan), and pyruvic acid (100 µg/ml, final concentration; Calbiochem, San Diego, California).[17] This medium was designated $F_{10}P$. After adjusting the pH to 7.0 with 10N HCl, it was filter sterilized (Nalgene, 0.2 µm) and heat-inactivated at 56°C for 45 min. prior to storage at 4°C. Before treatment, the cells were "cleansed" (removal of $TK^{-/-}$ spontaneous mutants) by growing them first in $F_{10}P$ supplemented with thymidine, hypoxanthine, methotrexate (Lederle Laboratories, Pearl River, New York), and glycine (THMG medium) followed by growth in $F_{10}P$ supplemented with thymidine, hypoxanthine and glycine (THG medium).[17] For treatment, the cells were centrifuged and resuspended in F_5P (Fischer's medium containing only 5% horse serum).[17] Cell cultures to be cloned were diluted in cloning medium (CM).[17] This was prepared by adding a Noble Agar (Difco, Detroit, Michigan) solution at 60°C to F_o (Fischer's Medium without horse serum or pyruvic acid; pH 6.8) at 37°C supplemented with 20% horse serum (see above) and 220 µg/ml of a filter sterilized solution of pyruvic acid in water. The final concentration of agar in CM was 0.37%.

Originally, some of the CM contained BUdR (A grade, sodium salt; Calbiochem, San Diego, California) at a final concentration of 50 µg/ml as a selective agent for $TK^{-/-}$ cells.[17] The mutants arising on these plates were usually obscured by a background haze of nonmutant cells. The colonies on these plates were impossible to count on the automatic colony counter (see section on the Conduct of the Assay). Therefore, a new selective agent, TFT, was employed, (Sigma Chemical Co., St. Louis, Missouri) at a final concentration of 1 µg/ml.[15] The use of this agent eliminated the heterogeneity of the background allowing the use of the automatic colony counter to determine the numbers of colonies on these plates.[47]

All of the Fischer's media contained penicillin (31 µg/ml, final concentration; GIBCO) and streptomycin (50 µg/ml, final concentration; Sigma) in an effort to eliminate bacterial contamination. All media were stored at 4°C for up to two months without loss in their ability to support the growth of L5178Y cells in all aspects of the assay procedure (treatment, expression, cloning, *etc.*)

Positive control compounds were EMS (Eastman Chemical Co., New York) for the nonactivation experiments and B(a)P (Gold Label®, Aldrich Chemical Co., Milwaukee, Wisconsin) and/or 2-AAF (RFR Corp., Hope, Rhode Island) for the activation studies. (Experiments to find a negative control compound for this assay are in progress and shall be completed in the near future.) Initially, solutions of each chemical were freshly prepared for each experiment by dissolving them in DMSO (Grade I; Sigma). Later, it was determined that the chemicals used in the activation experiments could be stored frozen (-80°C) and reused for up to a year without a loss in activity.

Storage and Routine Growth of Cells

Late log phase, $TK^{+/-}$ cells (5 x 10^5 cells/ml) were concentrated (by centrifugation) ten-fold in freshly prepared freezing medium[17] and 1 ml was dispensed into 2 ml capacity Costar Pro-Vials® (Cooke Laboratory Products, Alexandria, Virginia). Eight Pro-Vials® were placed in the BF-5 Biological Freezer (Union Carbide Corp., New York) which was set at ring position A in a liquid nitrogen tank (Linde Corp., a division of Union Carbide, New York) for two hours. After this time period, the Pro-Vials® were immersed in liquid nitrogen for long-term storage.

To grow $TK^{+/-}$ cells starting from a frozen liquid nitrogen culture, the following procedure was employed.[17,36,52] One ampoule of cells was rapidly thawed at 37°C without agitation. Using a sterile Pasteur pipette, the contents of the ampoule were carefully layered onto the bottom of a 125 ml round bottom flask containing 50 ml of $F_{10}P$ medium. The contents of the flask were gassed for 10 sec. with a 5% CO_2-95% air mixture and statically incubated for 24 hours at 37°C. Following this incubation, the number of particles (cells) in the culture was determined using a Coulter Counter® (Model Z_B; Coulter Electronics, Hialeah, Florida).[17] This was done by incubating the cells in trypsin to ensure the breakdown of binding proteins which exist between cells (0.1% trypsin from GIBCO in Dulbecco's Phosphate Buffered Saline without Calcium and Magnesium). Twenty milliliters of this culture were placed in a 50 ml Corning disposable centrifuge tube (Corning Glass Works, Corning, New York) gassed and incubated at 37°C in a tissue culture Rollordrum® (Model TC7; New Brunswick Scientific Co., New Brunswick, New Jersey, height 2 3/8 inches from the support stand, 40 rpm). Incubation was continued until the culture reached 3 x 10^5 cells/ml (about three days after removal of the cells from liquid nitrogen) at which time the culture was diluted (split) to 1 x 10^4 cells/ml with $F_{10}P$ and 20 ml per 50 ml centrifuge tube (after gassing) was incubated on the Rollordrum® as described above. The culture was grown until the titer was 3 x 10^5 cells/ml (about two days) at which time it was split as described above. For routine maintenance of the cells, cultures were split on Monday and Wednesday to 1 x 10^4 cells/ml and on Friday to 5 x 10^3 cells/ml. Under these conditions, the cells always grew with a generation time of 8-10 hours.

At the present time, a continuously growing cell culture is maintained until some "problem" arises. For example, generation times were not 8-10 hours, the spontaneous (background) MF was not in the range of 20-50 x 10^{-6}, the occurrence of equipment failure, and over long holiday periods.

When a culture had been growing for some time (2-3 weeks), the agreement of the Coulter Counter® (counts live and dead cells, *i.e.*, particles) determinations was compared with that made by the trypan blue staining procedure[39] (dead cells absorb the stain while live ones do not). These data revealed the two procedures differed by 5-15% (the Coulter® numbers were always greater than the trypan blue numbers) which was consistently shown to be the number of dead cells in the culture.[33] Thus, we were confident that the Coulter Counter® could be used to measure viable counts of a growing population of L5178Y cells.

Source, Preparation, and Storage of Microsomes

Originally the microsomal preparation was obtained with the assistance of Drs. D. Towle and F. Leftwich of the Department of Biology at the University of Richmond. However, for the past few years, we have used a commercial preparation (Litton Bionetics, Maryland). No significant differences (protein, biological activity of control compounds, *etc.*) have been found in S9 from either of these sources.[46] The inoculation procedure using Aroclor® 1254 (Monsanto Co., St. Louis, Missouri) and the preparation of the microsomes from the livers of male, Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) have been described.[2,4,31] The microsomes were stored at -80°C until used in the assay. Microsomes prepared and stored in this manner were shown to retain activity in this system for at least ten months.[34] The S9 preparation generally contained 35-40 mg/ml of protein[48] as determined by the Lowry method[29] using bovine serum albumin (fatty acid free; Sigma) as a reference standard.

Preparation of S9 Mix

The S9 mix was prepared according to Clive.[15] In the experiments presented here, 100 ml of F_0 containing 1500 mg of isocitric acid (Sigma) and 800 mg of NADP (Sigma) was filter sterilized (Nalgene, 0.2 µm) and the resulting cofactor mix stored on ice until added to the S9. The S9 was thawed in ice water and then 1 ml was mixed with 3 ml of cofactor mix; this preparation was termed S9 mix.

WSC Preparation

Small batch (<15 g), ECT or IT WSCs were prepared by the members of Project Charge Number 6908.[36,37,42] These WSCs were stored solvent-free at -80°C for about two days prior to testing in the assay. For use in an experiment, all of the WSC was dissolved in DMSO and, if necessary, further dilutions of this stock (generally 30-60 mg/ml) were prepared in DMSO. Fifty microliters of WSC "solution" were used per treatment tube.

Conduct of the Assay

Generally, the method of Clive and Spector[15] was employed. For the purposes of this report, an *exact* description of how the assay is conducted at PM is presented below.

On the Thursday preceeding Monday's treatment, the routinely growing $TK^{+/-}$ cells (see above) at about 3×10^5 cells/ml were diluted to 5×10^4 cells/ml in "cleansing medium" ($F_{10}P$ plus THMG). The methotrexate in this medium kills $TK^{-/-}$ cells, which arise spontaneously in the culture, because the drug "shuts down" the only pathway by which $TK^{-/-}$ cells can make thymidylate. In $TK^{+/-}$ cells, death does not result from the drug because a second biochemical pathway is utilized to produce this essential DNA constituent.

After 24 hours of growth (about two generations), the cells were diluted to 5×10^3 cells/ml in $F_{10}P$ plus THG medium for the weekend. This step is critical because even though $TK^{+/-}$ cells are not killed by methotrexate, their ability to make thymidylate is decreased by ~50%. Addition of THG to the growth medium helps to overcome the effects of the drug by supplying needed thymidine.

On Monday (the treatment day), the cells were counted and concentrated to 1×10^6 cells/ml in F_5P medium. Six milliliters of these cells were placed in a 50 ml Corning centrifuge tube to which was added 4 ml of ice cold F_0 medium (nonactivation experiments) or 4 ml of ice cold S9 mix (activation experiments). Each tube received 50 µl of DMSO (final concentration of 0.5%) either as a blank (solvent control) or containing the agent to be tested. This concentration of DMSO has little if any effect on the L5178Y cells (data not shown).[45] The contents of the tubes were gassed and incubated for four hours at 37°C in the Rollordrum®. Following incubation, the tubes were centrifuged (500 x g for ten minutes at room temperature) and the cells resuspended in 10 ml of $F_{10}P$ medium. This washing procedure was done two more times with the cells finally being resuspended in 20 ml of $F_{10}P$ medium. The purpose of this procedure was to remove the residual treatment agent from the cells.

The cells at an initial concentration of 3×10^5 cells/ml were grown for three days using conditions as described above. This phenotypic lag period or expression time was necessary in order to "fix" the mutation(s) in the induced, $TK^{-/-}$ mutants and to allow these same mutants to "dilute out" their intracellular pools of TK enzyme. Each day during the expression period, the cells were counted and, if necessary, diluted to 3×10^5 cells/ml with $F_{10}P$ medium.

On the third day of the expression period (usually Thursday), after the cells were counted and diluted, they were cloned in soft agar. Fifteen milliliters of cells (3×10^5 cells/ml) were centrifuged as described above and resuspended in 1.5 ml of $F_{10}P$. Soft agar (CM containing 0.37% Noble agar) was prepared as previously described and stored in desired aliquots (depending on the dilutions needed) in a 37°C water bath. The cells (3×10^6 cells/ml) were diluted 10^{-2} in the soft agar solution and the dilution vessels were labeled "TFT". These

diluted cells were further diluted 10^{-4} in soft agar so that the final concentration was three cells/ml. After the 10^{-4} dilutions, a TFT solution was added to the cells in the containers labeled "TFT." TFT is the selective agent (final concentration = 1 µg/ml) for $TK^{-/-}$ cells. [It is a thymidine analog which can be utilized by $TK^{+/-}$ cells (because they have TK) but not by $TK^{-/-}$ cells. When TFT is incorporated into $TK^{+/-}$ cells, they die.] Four, 20 ml petri plates (Optilux, 15 x 100 mm; Falcon, Oxnard, CA) were prepared containing the 10^{-2} dilution of cells. [These were the mutant plates; *i.e.*, colonies arising here were composed of TFT-resistant TFT^r or $TK^{-/-}$ cells.] Four, 20 ml petri plates were also prepared containing 10^{-4} dilution of cells (or 10^{-6} dilution of the initial cell concentration). [These were the viable count plates, *i. e.*, colonies arising here were composed of $TK^{+/-}$ cells.] The plates were allowed to solidify at room temperature for 15 minutes after which time they were placed in a 5% CO_2-95% air, humidified (90-95% relative humidity) incubator at 37°C.

After eight days of incubation, the colonies on all of the plates were counted on the BioTran II automatic colony counter (New Brunswick Scientific, NJ) using machine settings especially established for this assay system.[44,56] The following were strictly adhered to: power--on; image--positive; light--bottom; sensitivity--6.25; area--474.0 mm; compensation--12; size discriminator--off; the F stop and the focus (which are normally set by the Brunswick service representative) should be at 5.6 and 7.0, respectively. Each plate was counted three times rotating the plate 120° between counts. The median value of the three determinations was assigned to each plate.

When BUdR was used as the selective agent, all of these plates (and for convenience the viable count plates) had to be counted by hand.[36,37,46] With the advent of TFT, this was no longer necessary.[47] Occasionally, we wished to count some of the TFT plates by hand due to high numbers of mutant colonies which the BioTran would underestimate. In addition, clumps and debris in the agar, which would appear like colonies and thus be counted by the BioTran, would also necessitate counting the plates by hand. The following procedure was used for hand counting plates. The plates were placed over a grid which was covered with a red filter glass (0.50C; Ciba-Geigy Co., NY). To further aid in counting the colonies, the plates were illuminated from above with a Luxo Magnifier Lamp (LFM 1/A; Luxo Lamp Corp., Port Chester, NY) equipped with a 2X lens.

From previous experiments,[43] the mathematical relationship that exists between hand and BioTran counts for the plates containing the selective agent is as follows:

$$\log_e \text{ MF TFT}^r \text{ colonies} = 0.268 + 0.990 \ (\log_e \text{ MF BUdR}^r \text{ colonies})$$

$$\log_e \text{ MF BUdR}^r \text{ colonies} = 0.472 + 0.864 \ (\log_e \text{ MF TFT}^r \text{ colonies})$$

No mathematical relationship was needed for the viable count plates done by hand or by machine since the numbers from either determination were similar.[44,56]

When experiments were conducted in this manner, the spontaneous MF was in the range of 20-50 x 10^{-6} which is consistent with other investigators using these cells.[1,15,27,30,41] [This will be discussed in greater detail in the RESULTS AND DISCUSSION section of this report.]

Analysis of Data

Attempts were made to analyze the data using linear regression, exponential and quadratic equations. Mr. John Tindall finally decided that the dose-response results best fit the following exponential equation: $y = ae^{bx}$ where y is the MF x 10^{-6}
x is the dose in micrograms
a is the MF x 10^{-6} at dose = 0; *i.e.*, the spontaneous MF
b is a constant related to the activity of the agent;
a larger *b* implies a more active agent. A *b* value was calculated for each dose of agent that was tested. The *b* values from different experiments were averaged for the same dose. The median was obtained for all the doses from a given agent and an ANOVA[21] was done using these medians in an effort to determine if the agents tested were different from each other. Since *a* was fairly constant for this system and, generally, the agents were tested at the same dose (*x*), these terms were eliminated from the above equation. In addition, an attempt was made to try and produce more consistent variations between MFs. Thus, taking the natural logarithm of both sides of the original equation yields $b = \log_e \text{MF}$.

One of the objectives of this paper was to establish the minimal acceptable criteria for testing samples in this assay. In order to determine a base line response for the spontaneous (solvent treated cells) and positive control treated cells, an analysis (ANOVA) was conducted on data obtained over a period of 2.5 years. These findings will be discussed in the RESULTS AND DISCUSSION section of this report.

Waste Disposal and the Good Laboratory Practices Act

All known hazardous and/or potentially hazardous waste was disposed of in the manner described in Appendices A (solid hazardous waste disposal procedure) and B (liquid hazardous waste disposal procedure). Although these are not identical to those described in references 22 and 23, they are "a step in the right direction." Modifications will be implemented as needed.

RESULTS AND DISCUSSION

The Response of a Pure Chemical Without Microsomal Activation

EMS (a direct-acting mutagen)[13,15] was used as a positive control for the establishment of the assay in our laboratory. A dose-response relationship was observed for EMS (see Table 1 and Figure 1). When the number of mutants (Column 9, Table 1) was compared to the dose (Column 1, Table 1), the total number of mutants increased as the dose was increased. At 124 µg/ml of EMS, a significant increase in the number of mutants over background was observed (Column 13, Table 1) with low cell killing (Column 10, Table 1).

These data suggest that EMS does not select for existing mutant cells in the population, but rather that it induced a forward mutational event[26] (*i.e.*, $TK^{+/-}$ competent cells → $TK^{-/-}$ deficient cells). At higher doses, a plateau in the dose response was not often seen because of the increased toxicity. It was evident that EMS induced a 7 to 176-fold increase in the MF when the number of mutants per survivor was compared to the solvent control treated cells (see Table 1).

The Response of Pure Chemicals With and Without Microsomal Activation

It became evident in the early stages of assay development that incorporation of mammalian microsomes was necessary in order to obtain the maximum activity of a WSC.[35] B(a)P (a compound which requires metabolic activation in order to exert its effects[15]) was initially chosen as the positive control.

In the lower half of Table 2, the response of B(a)P in the absence of microsomes shows little or no difference over that of the solvent control with regard to MF, IMF, or percent survival. However, when microsomes were added, a dose-response relationship was obtained (upper half of Table 2). When these data were plotted as previously described, there was a significant increase in the number of induced mutants over that observed without microsomal activation and as expected, the percent survival was decreased (Figure 2).

It was suggested[52] that in addition to B(a)P, 2-AAF should also be used as a positive control in activation experiments. This was due to interexperiment variation which was greater with 2-AAF than B(a)P[52] and it was felt that 2-AAF was measuring the variation in the microsomal activation mix.

The data in Table 3 (and Figure 3) show the response of 2-AAF in this system. As with B(a)P, 2-AAF was very active in the presence of microsomes (upper half of Table 3). Unlike B(a)P, however, 2-AAF seemed to exhibit weak activity in the absence of microsomes (lower half of Table 3). This phenomenon has also been observed by others[38,51] and suggests that these cells possess small amounts of the enzymes necessary for activation of this compound.

The Response of a WSC (Kentucky Reference, 2R1) with and without Microsomal Activation

Dose-response experiments without microsomal activation (lower half of Table 4) revealed only slight activity. In addition, the extreme toxicity of the WSC in the absence of microsomes severely limited the dose range used (about 50-100 µg/ml). Therefore, a microsomal activation system was necessary to obtain maximal WSC activity.

Addition of microsomes to the WSC in the treatment tube caused a reduction in toxicity which, in turn, expanded the dose range studied (upper half of Table 4). The corresponding figure (Figure 4) shows that a dose-response relationship was obtained with regard to IMF and percent survival.

Objectives of the Statistical Analyses

Over the last 2.5 years, the agents just described have been tested many times as part of the controls for each experiment. Each time an experiment was conducted, the control data just described were *subjectively* compared to those of the previous two or three experiments. If found to be similar, the present experiment was said to be acceptable and the test data from it were analyzed, firm conclusions were made, and future plans were decided upon.

For several reasons, it was deemed necessary to examine in detail all of the historical control data:

1. In some experiments, the control data were significantly different from previous data. Why?

2. What effect did changing the known assay variables (cell stock, microsomal preparation, stock of control compound and selective agent) have on the activities of the control agents?

3. Some objective procedure was necessary to determine the *validity* of an experiment so that experiments would be comparable.

The effect of each of the known variables mentioned in 2 above was determined in a concurrent experiment. [The usual procedure *versus* one modification of the procedure in the same experiment.] The data were statistically analyzed to determine if the modification was altering the biological activity of the controls. These experiments were usually performed only once. Data from experiments conducted over the past 2.5 years might yield different conclusions.

The Generation of the Data Base

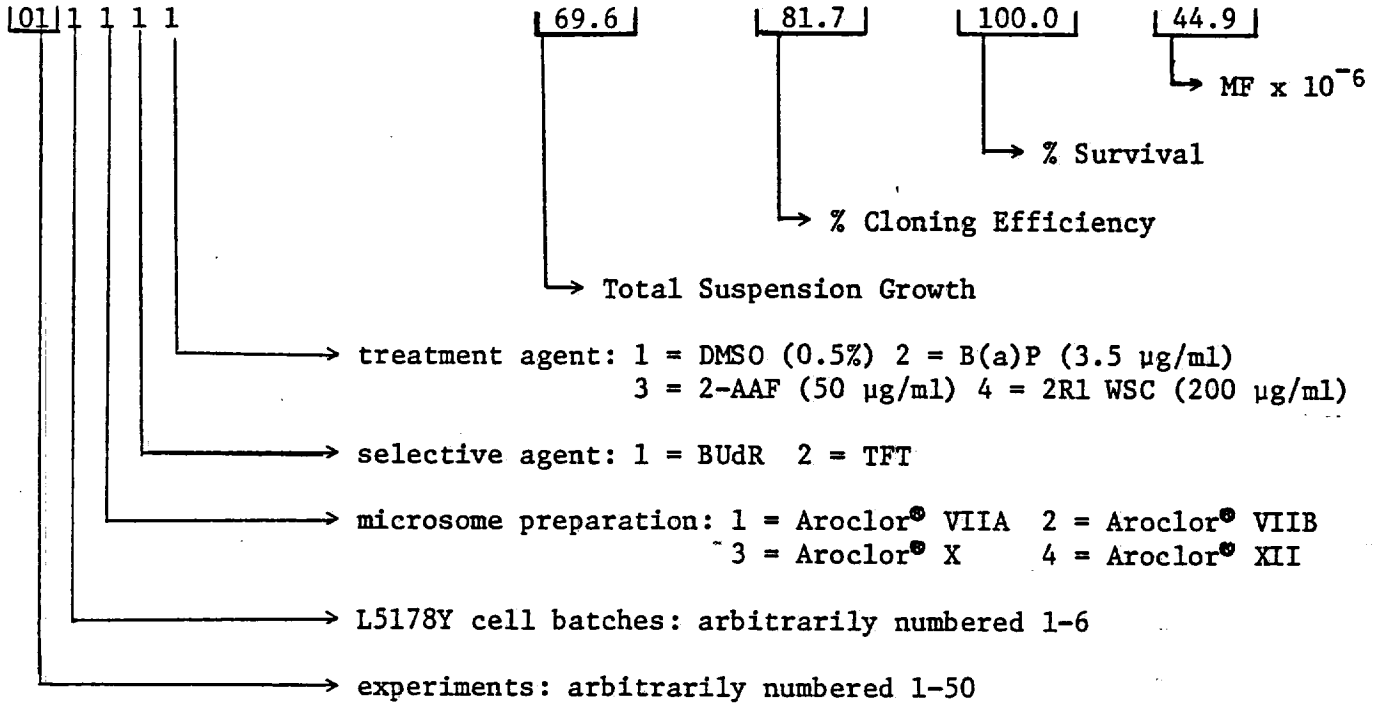
It was decided to examine the control data (described above) from all of the experiments that had been done in this assay system and try to place them into a group not having any known

variables. The controls examined were DMSO, B(a)P, 2-AAF and 2R1 WSC with microsomal activation and DMSO and EMS without microsomal activation. The first observation was that at the time this was done (August 1979) enough data for statistical analyses existed only for those agents that needed microsomal activation. Therefore, it was decided to analyze data from DMSO, B(a)P, 2-AAF, and 2R1 WSC.

Analyses of the 50 experiments utilizing these four agents revealed that only ten experiments could be placed in a group without any known variables. Statistical conclusions based on such a small number of experiments would be meaningless. Closer examination of the 50 experiments revealed that they could be placed into groups having only one of the known variables that were previously mentioned. Statistical analyses could then show if the known variables were influencing the results. The largest known variable was the experiment number followed by the cell batch number. Sometimes new cell cultures were started even though no problems existed with the current cell culture. The effect(s) of using continuously growing cells for long periods of time in this assay system are unknown. The third variable was the microsomes. Significant quantity of one microsomal preparation was not available for experiments covering 2.5 years. Over the course of these experiments, two selective agents (see MATERIALS AND METHODS) were employed which constituted the fourth variable. The last variable was the treatment agent itself (either DMSO, B(a)P, 2-AAF, or 2R1 WSC).

The next challenge was which assay parameter(s) to analyze to determine if these known variables were influencing the data. Arbitrarily, it was decided to use everything measurable in a given experiment. The measurable assay parameters were: the total suspension growth in liquid culture during the expression period (*e. g.*, column 5, Table 1); the cloning efficiency in soft agar of the treated cells *versus* the number of cells plated (*e.g.*, column 7, Table 1); the percent survival (*e.g.*, column 10, Table 1); and the MF (*e.g.*, column 11, Table 1).

With the assistance of Mr. John Tindall, these assay parameters were described in table form (see Appendix C). To explain Appendix C, record number 1.000 from the Appendix will be used as an example.



The Statistical Analyses of the Data Base

The data presented in Appendix C were analyzed using a series of ANOVA[21] computer programs written by Mr. Tindall (see Appendix D). Admittedly, Appendix D is awesome, but it forms the basis for one of the major objectives of this report, and thus, some attempt must be made to understand it.

First, ANOVA's were done for experiments within the same class. For example, on page 1 of Appendix D is given the ANOVA for the suspension growth (SUSPGR) of cells in experiments (1-4) which comprise class 111. These four experiments were conducted with the following variables held constant: L5178Y cell batch number 1, microsome preparation VIIA and BUdR which was used as the selective agent. In these four experiments, the controls that were tested were DMSO (the solvent control) and B(a)P. Thus, suspension growth data appear only for these agents while the data for the other agents is given as 0.00. The results of the ANOVA for class 111 are given in the probability column (PROB) near the bottom of page 1, Appendix D. They show that there was a significant difference between the agents tested. (The number 0.0049 roughly means that in five experiments out of 1000 experiments these two agents will be the same, *i. e.*, produce the same random error or variation about a mean of their respective suspension growth data. Since this was not a very high probability that the two agents were the same, they must be different.)

This result was not unexpected since DMSO and B(a)P are known to be different agents. At the concentrations employed, DMSO was not active[45] while B(a)P was active. This activity was reflected in the total suspension growth.

The next number in the probability column (0.0479) on page 1 of Appendix D shows that there was a significant difference among experiments. This was unexpected since there should be no significant differences among experiments comprising class 111 in which there were no differences in known variables (cell batch, microsome preparation, and selective agent). The reason for this difference(s) is unknown, however this observation will be discussed in the next section of this report.

These analyses were continued for all the different classes (different variables) from the 50 experiments in the data base. On page 7 of Appendix D, appears the analysis for the last suspension growth class (642). The next task was to analyze the DMSO suspension growth (page 7, Appendix D) and the B(a)P suspension growth (page 8, Appendix D) across the different classes. For example, class 111 was compared to class 211 which differed only by the L5178Y cell batch number. This would indicate if any of the variables which were known to exist between classes (cell batch number, microsomal preparation, *etc.*) were significantly influencing the suspension growth results. As an example, using the DMSO analysis found near the bottom of page 7 in Appendix D, one observes a probability of differences between classes (listed below the ANOVA table) of five out of 10,000 experiments done. Since this was a very low probability that these classes were different, the conclusion reached was that the three known variables (L5178Y cell batch, microsome preparation and selective agent) were not significantly influencing the DMSO suspension growth data.

In like manner, the remaining assay parameters were statistically analyzed. However, the paucity of 2-AAF and 2R1 WSC data within and across the various classes necessitated some statistical analyses (mean and standard deviation) by the authors. In addition, Mr. Tindall analyzed the natural logarithm of the MF in an effort to determine if this data transformation would produce a more uniform variation observed in the MFs.

In the cursory description given here of Appendix D, the reader should not attempt to grasp all the potentially important information. For example, one can use the residual mean square error from the ANOVA across classes to rank those classes and perhaps suggest which classes have a tendency to be different from others.

Conclusions Derived from the Analyses of the Data Base

1. The best parameter for measuring that differences existed between agents, that no differences existed between experiments, and that there was no interaction between these two was the MF. This was closely followed by the $\log_e$ MF and percent survival. It is a well known fact[3,15,40,49] that the percent survival and MF (and by definition the $\log_e$ MF) are closely related to the biological activity of many test systems. One possible interpretation of this finding was that the suspension growth and percent cloning efficiency were not good predictors of biological activity in this system.

2. Regardless of the parameter, no class was better than another for measuring differences between agents, differences between experiments, and/or interaction between these two. The ability of a class to predict differences was heavily dependent on the number of experiments within that class. The larger the number of experiments per class, the better able it was to detect differences between agents, no differences between experiments, *etc.*

3. Of the variables examined (L5178Y cell batch number, microsome preparation, *etc.*), there was a tendency for a new batch of L5178Y cells to yield higher activity values for some of the parameters (higher MFs, lower percent survivals, *etc.*). However, to prove this would require a much larger data base or at least one involving two or three times as many new cell batches. Of the remaining variables, none seemed to significantly influence any of the parameters that were measured.

4. The natural logarithm of the MF did produce a more uniform variation between the MF values. For example, the variation (mean square error) in the MFs between experiments for DMSO and B(a)P (Appendix D, page 30) was 9.4. When the $\log_e$ MF was used (Appendix D, pages 37, 38), this value was considerably lower (2.2). This was predicted by Mr. Tindall and further strengthens the mathematical model which best fits the dose-response curves obtained in this system; *i. e.*, $y = ae^{bx}$ (see MATERIALS AND METHODS).

5. As mentioned in the previous section, using these agents the largest amount of variation was observed between experiments. The reason(s) for this is unknown. It does suggest that a comparison between two agents is best done within the same experiment.

6. One part of the analyses from Appendix D can be summarized as seen on Table 5. The mean and standard deviation are given for each agent using the different known variables. The usefullness of this table will be presented below.

Minimal Acceptable Criteria for the L5178Y Assay

Introduction

It would seem as though the data found in Table 5 would suffice as criteria for determining whether a given test was valid. All one would have to do was compare the control data from the experiment in question with that obtained from the data base (Table 5). If the numbers were within the designated ranges, the experiment was acceptable and the other noncontrol data were usable. If not, the experiment was unacceptable.

One immediate problem arising from casual use of Table 5 is should all of the control data agree with that of the data base? One can address this question and gain some insight into the value of Table 5 by considering the following hypothesis. All 50 experiments listed in Appendix D and summarized in Table 5 were *subjectively* judged to be valid experiments. Therefore, using Table 5, all the parameters for the controls should agree with the data base. One could construct a table to show this.

DMSO	SUSPGR	% CLNEF	% SURVI	MUT FRQ
Experiment 1	+	+	+	+
Experiment 2	+	-	-	+
Experiment 3	-	+	+	+

etc.

Here a plus (+) means the parameter for that experiment agreed with the data base while a minus (-) would imply that it did not.

Thus, in an objective way, Table 5 could be used to determine if an experiment was acceptable. The findings from such an effort were as follows:

a) None of the 50 valid experiments agreed perfectly with the data base.

b) 90% of the DMSO values gave pluses for all the parameters that were measured.

c) 66% of the B(a)P values and only 50% of the 2-AAF values gave pluses for all the parameters that were measured.

Clearly, these results showed that all of the data in Table 5 alone were insufficient in defining a valid experiment. Therefore, as presented in the remaining sections of this report, a more detailed examination of the assay was necessary to define minimal acceptable criteria for an experiment.

CRITERIA FOR CONDUCTING TEST

Uniform standards for conducting the assay at PM must be established to minimize misinterpretations of test results and permit intralaboratory comparisons.

Solvent Control

An untreated or a solvent control (usually DMSO) will be included in each assay to determine the solvent effect on cytotoxicity and activity. The final concentration of DMSO in the treatment tube will range between 0.5 and 1% unless otherwise warranted for special compounds.

Positive Control(s)

1. Activation

Two positive control compounds--B(a)P at 3.5 µg/ml and 2-AAF at 50 µg/ml per treatment tube--which require metabolic activation are concurrently analyzed in each test to monitor the proper functioning of the system. The inclusion of both compounds seemed repetitious since the purpose here is only to monitor the microsomal activation system. It is recommended that B(a)P with its more stable mutagenic frequency be the routine positive control compound at PM.

For standardization, a stock solution of B(a)P at 0.7 mg/ml should be prepared in DMSO and stored at -70°C in a brown bottle prior to testing. Ideally, the preparation of a new stock solution will occur before depletion of the old stock to allow parallel testing of both stock solutions. Whenever possible, duplicates of these samples will be tested to check intraexperimental variation.

WSC from the Kentucky Reference Cigarette (*i.e.*, 2R1) will be tested in all experiments involving tobacco products as a control for the smoke collection process. A single dose of freshly prepared 2R1 WSC in DMSO will be used at a final concentration of 150 µg/ml in the treatment tube.

2. Nonactivation

Complete characterization of some agents will necessitate conducting the nonmicrosomal (-S9) assay. When this portion of the assay is performed, a solvent and suitable positive control will be included. EMS, a direct acting agent, at a final concentration of 686 µg/ml per treatment tube, diluted in DMSO for adequate sample size, is the recommended positive control.

Whenever possible, EMS will be included as a control in assays containing activation as a primary monitor of cells and media.

Negative Control

At present, a negative compound is not routinely included during testing because none is available and our search for a truly negative compound in this system is being continued.

Microsomal Activation and Evaluation

Metabolic activation will be included in testing all complex mixtures (*e.g.*, WSC), solvents, and other pure chemicals. The S9 fraction which is commercially available will be used after proper evaluation.

Evaluation of commercial preparations of microsomes (S9) is essential for allowing transitions between different lots of microsomes without affecting previous results. First, sterility checks for contamination will be conducted on all preparations.[48] Second, protein determinations will be performed in-house.[29] Third, comparison of different lots of microsomes shall be conducted by testing them concurrently at equal protein concentrations. The test agents for this comparison shall be: the ten Model I WSCs at 150 µg/ml; B(a)P at 3.5 µg/ml; and 2-AAF at 50 µg/ml (all final concentrations). The activities of the WSCs will be evaluated statistically by linear regression to detect any change in their rank order.[46] If little or no differences exist between two microsomal (S9) preparations, then the new S9 preparation will be routinely used when the current lot is depleted.

Dose Selection

The selection of doses for pure chemicals will depend upon their solubility in aqueous solution and/or organic solvents, mainly DMSO. A broad dose range starting with the upper limits of solubility in the stock solution and decreasing at 2 - 10 fold increments will be tested. Treatment and expression will involve a large number of samples; however, only those doses with an observable 30-95% reduction in total growth at the end of the expression period will be cloned. Repeat experiments will be conducted with narrower doses selected to bracket a suspect dose or to provide additional information.

Dose selection for WSCs, fractions, TPMs, and/or pyrolysates will initially require the fresh preparation of stock solutions in DMSO. Whenever necessary, dilutions of the stock solution representing at least three doses and preferably five will be used for treating the cells. Only those doses with 5-70% survival at the end of the expression period will be cloned in soft agar. As indicated above, repeat experiments may be necessary. Known agents (*i.e.*, Model I cigarettes) with established dose-response curves at PM may be tested at a single dose during microsomal evaluation.

Procedural Modification

Preliminary studies using the solvent and positive control compounds shall be performed to ascertain the feasibility of making a procedural change. Evaluation of the proposed change with the standard procedure will be conducted in simultaneous experiments. The definitive experiment will involve testing the following: DMSO, B(a)P, 2-AAF (at doses previously described) and a fresh IT WSC preparation of 2R1 at 150 and 200 µg/ml. The data will be analyzed according to the criteria described in the assay acceptance section of this report (see below).

CRITERIA FOR ASSAY ACCEPTANCE

The L5178Y assay will be considered acceptable for evaluation when the following criteria are satisfied. If necessary, an assay may be repeated to fulfill some of the acceptance standards.

Solvent Control:

Background mutation frequency provides an estimate of the number of mutants which were either pre-existing or arose spontaneously in the cell population during the experiment. The background should average 32.5 ± 23, however, assays with background MF outside this range are not necessarily unacceptable but will be evaluated on an individual basis because a high background can mask a weak mutagenic response.

The total suspension growth which measures reduced growth rate and cell death should average around 80 ± 28 for the solvent control. The cloning efficiency for the solvent control shall range between 53 and 105% (Table 5). Suspension growth and cloning efficiency outside the acceptable range will not, by itself, be sufficient evidence to invalidate an entire experiment. As previously discussed, these parameters have been shown to be unreliable predictors of activity in this assay.

Positive Control

The normal range for the five parameters of the positive control compounds has been determined and appears in Tables 5 and 6. Data outside the acceptable range will be suspect and their acceptance will be contingent upon the overall results. Such experiments will be judged by all experienced L5178Y researchers at PM with a majority opinion necessary for assay acceptance.

Experimental Compounds

An experimental compound will be considered acceptable for evaluation if its percent suspension growth does not exceed 70% of the solvent control growth. A complete survival range (1 - ∿70%) will be necessary for evaluating the presence (or absence) of activity. However, survivals below 10% are suspect because MF increases as survival decreases.

A minimum of two out of four petri dishes (to allow for contamination) will be required to determine both the mutant and viable colony counts. For acceptance of the experiment, the MF shall be derived from at least two different doses of the test compound. However, a single dose may be acceptable if it is the maximum dose tested at ≥ 10% survival.

CRITERIA FOR EVALUATING TEST RESULTS

An assay will be considered for evaluation of test results if the criteria given under assay acceptance are satisfied. The microsomal (+S9) and nonmicrosomal (-S9) assays are sometimes performed concurrently, but each portion is an independent entity and should be evaluated as such. A test result may be classified as either positive, negative, or inconclusive.

POSITIVE RESPONSE

The minimum criteria necessary for demonstrating a positive response will be a two-fold increase in mutation frequency above the spontaneous background at survivals ≥ 10%. A dose response in either the number of TFT^r colonies/µl or the mutation frequency/10^6 survivors should be observed for two or more doses.

Under special circumstances, other criteria may be considered in determining a positive response. An increased MF of twice the background for the same dose in multiple experiments will be called positive. Confirmation of increases that are less than twice background (≥ 10% survival) from a single dose will be required and the experiment will be repeated over a narrow dose range in an effort to obtain a dose-response curve.

Quantitative differentiation between the activities of two positive agents in this bioassay may necessitate the use of ANOVA or other statistical methods.

Negative Response

A substance will be adjudged inactive (negative) under these test conditions if the following criteria are met: 1) at the maximum dose tested survivals should be at or near 10%; 2) there is no observable dose response in mutation frequency; 3) there is no significant increase in MF above background (not ≥ 2x) at 10% survival.

Inconclusive

Additional testing will be necessary to reach a conclusion regarding the activity of a substance in this category. An agent which produces no cytotoxicity or any assay which contains incomplete data (*e.g.*, no positive control) will be considered inconclusive.

If there is any doubt as to whether or not the results of a given experiment are positive or negative, statistical analysis of the data using ANOVA or any other appropriate method will be acceptable in helping to reach a conclusion.

CONCLUSIONS

1. This report has accurately described the handling of L5178Y cells and the conduct of the assay at PM.

2. A description for evaluating or analyzing the experimental data has been given (biologically and/or statistically).

3. An attempt was made to rigorously define the criteria necessary for a valid experiment.

4. Any changes or revision of this standard operating procedure (SOP) will be documented and maintained as an addendum to this report.

5. The "bottom line" is that it is not objectively possible to use previously obtained positive control data to determine if future experiments are acceptable.

ACKNOWLEDGMENTS

We are indebted to Mr. John Tindall of the Technical Planning and Information Division for his excellent statistical analyses of our data. We thank Mr. Ralph Hellams and the other members of the condensate preparation group of Project Charge Number 6908 for their assistance. In addition, we owe a debt of gratitude to Mr. William F. Kuhn and Dr. Robert A. Pages for their critical review of this manuscript. Finally, we appreciate the special efforts of Mrs. Nancy Peters in typing this manuscript.

REFERENCES

1. Amacher, D. E.; Paillet, S.; Ray, V. A. Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. I. Application to genetic toxicological testing. Mutation Research. 64:391-406; 1979.

2. Ames, B. N.; McCann, J.; Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Research. 31:347-364; 1975.

3. Asquith, J. C.; Paice, C. C.; Reading, A. H. An effect of viable cell proportion on the recovery of clones of Chinese hamster cells resistant to 8-azaguanine and 6-thioguanine. Mutation Research. 54:355-366; 1978.

4. Booker, J.; Neal, W. K. The activity of ten whole smoke condensates representing models for high and low estimated biological activity in the *Salmonella typhimurium* assay. Special Report 76-129; 1976 August 4.

5. Clive, D. Mutagenicity of hycanthone and several of its analogs in L5178Y mouse lymphoma cells. Mutation Research. 2:216; 1973.

6. Clive, D. Recent developments with the L5178Y TK heterozygote mutagen assay system. Environmental Health Perspectives. 6:119-125; 1973 December.

7. Clive, D. Mutagenicity of thioxanthenes (hycanthone, lucanthone, and four indazole derivatives) at the TK locus in cultured mammalian cells. Mutation Research. 26:307-318; 1974.

8. Clive, D. Various parameters associated with mutagenesis. In: Asher, I. M. and Zervos, C., eds. Structural Correlates of Carcinogenesis and Mutagenesis. Washington, D. C.: The Office of Science, FDA; 1977 22-26.

9. Clive, D. Mutagenicity studies on chemical carcinogens. *In vitro*. 13(3):191; 1977 March.

10. Clive, D. A linear relationship between tumorigenic potency *in vivo* and mutagenic potency at the heterozygous thymidine kinase ($TK^{+/-}$) locus of L5178Y mouse lymphoma cells coupled with mammalian metabolism. In: Scott, D.; Bridges, B. A.; Sobels, F. H., eds. Progress in Genetic Toxicology. Amsterdam: Elsevier/North Holland; 1977:241-247.

11. Clive, D.; Bradlaw, J. *In vitro* testing for mutagenesis: Agreement (or lack thereof) among current methods and their correlation with animal studies. In: Berky, J. and Sherrod, C. eds. *In Vitro* Toxicity Testing. Philadelphia: The Franklin Institute Press; 1977:393-403.

12. Clive, D.; Flamm, W. G.; Machesko, M. R. Mutagenicity of hycanthone in mammalian cells. Mutation Research. 14: 262-264; 1972.

13. Clive, D.; Flamm, W. G.; Machesko, M. R.; Bernheim, N. J. A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Research. 16:77-87; 1972.

14. Clive, D.; Flamm, W. G.; Patterson, J. B. Specific-locus mutational assay systems for mouse lymphoma cells. In: Hollander, A. E. ed. Chemical Mutagens. Volume 3. New York: Plenum Press; 1973:79-103.

15. Clive, D.; Johnson, K. O.; Spector, J.F.S.; Batson, A.G.; Brown, M.M.M. Validation and characterization of the L5178Y/TK$^{+/-}$ mouse lymphoma mutagen assay system. Mutation Research. 59:61-108; 1979.

16. Clive, D.; McCuen, R.; Spector, J.F.S.; Piper, C. Specific gene mutations in L5178Y cells in culture. Gene Tox Technical Report for the U. S. EPA; submitted for publication.

17. Clive, D.; Spector, J.F.S. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Research. 31:17-29; 1975.

18. Clive, D.; Spector, J.F.S. Laboratory procedure for assaying specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. In: Kilbey, B. J.; Legator, M.; Nichols, W.; Ramel, C., eds. Handbook of Mutagenicity Test Procedures. Amsterdam; Elsevier Scientific Publishing Co.; 1977:161-173.

19. Clive, D.; Spector, J.F.S. Comparative chemical mutagenicity: Can we make risk estimates. In: de Serres, F. J. and Shelby, M., eds. Comparative Chemical Mutagenicity. New York: Plenum Press; 1979: in press.

20. Clive, D.; Voytek, P. Evidence for chemically-induced structural gene mutations at the thymidine kinase locus in cultured L5178Y mouse lymphoma cells. Mutation Research. 44:269-278; 1977.

21. Dixon, W. J.; Massey, F. J. Introduction to Statistical Analysis. New York: McGraw-Hill Book Co., Inc.; 1957.

22. Federal Register, Friday, November 19, 1976. Part II, Department of Health Education and Welfare, Food and Drug Administration. Nonclinical Laboratories Studies. Proposed Regulations for GLP. pp. 51206-51230.

23. Federal Register, Vol. 43, No. 243. Monday, December 18, 1978. pp. 48946-59028.

24. Fischer, G. A. Predictive tests in culture of drug-resistant mutants selected *in vivo*. National Cancer Institute Monograph. 34:131-36; 1971.

25. Fischer, G. A.; Lee, S. Y.; Calabresi, P. Detection of chemical mutagens using a host-mediated assay (L5178Y) mutagenesis system. Mutation Research. 26:501-511; 1977.

26. Ishii, Y.; Elliott, J. A.; Mishra, N. K.; Lieberman, M. W. Quantitative studies of transformation by chemical carcinogens and ultraviolet radiation using a subclone of BHK_{21} clone 13 Syrian hamster cells. Cancer Research. 37:2023-2029; 1977 July.

27. Jacobsen, E. D.; Krell, K.; Dempsey, M. J.; Lugo, M. H.; Ellingson, O.; Hench, C. W. Toxicity and mutagenicity of radiation from fluorescent lamps and a sunlamp in L5178Y mouse lymphoma cells. Mutation Research. 51:61-75; 1978.

28. Kruse, P. K.; Patterson, M. K. Tissue Culture Methods and Applications. New York: Academic Press; 1973.

29. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement and folin phenol reagent. Journal of Biological Chemistry. 193:265-275; 1951.

30. Macgregor, J. T.; Gould, D. H.; Mitchell, A. D.; Sterling, G. P. Mutagenicity tests of diflubenzuron in the micronucleus test in mice, the L5178Y mouse lymphoma forward mutation assay, and the Ames *Salmonella* reverse mutation test. Mutation Research. 66:45-53; 1979.

31. Malling, H. Dimethylnitrosamine: formation of mutagenic compounds by interaction with mouse liver microsomes. Mutation Research. 13:425-29; 1971.

32. Matheson, D.; Brusick, D.; Carrano, R. Comparison of the relative mutagenic activity for eight antineoplastic drugs in the Ames *Salmonella*/microsome and $TK^{+/-}$ mouse lymphoma assays. Drug and Chemical Toxicology. 1(3):277-304; 1978.

33. McCuen, R. W. PM Notebook No. 6825, p. 100.

34. McCuen, R. W. PM Notebook No. 7060, p. 55.

35. McCuen, R. W. Trip report to the laboratory of Dr. Donald Clive, Burroughs Wellcome Company, Research Triangle Park, NC, September 20-22, 1976. Memo to Mr. W. F. Kuhn; 1976 September 29.

36. McCuen, R. W.; Penn, J. M. The bioactivity of several whole smoke condensates in the L5178Y mouse lymphoma thymidine kinase mutation assay. Special Report 78-005; 1978 January 3.

37. McCuen, R.; Penn, J. L5178Y $TK^{+/-}$ mouse lymphoma assay--WSC activities of the Model I cigarettes. Memo to R. A. Pages; 1978 July 14.

38. McGregor, D. B. The apparent nonmicrosomal activation of 2-acetylaminofluorene to a mutagen *in vitro*. 11th Annual Meeting, Environmental Mutagen Society; 1980 March.

39. Merchant, D. J.; Kahn, R. H.; Murphy, W. H. The Handbook of Cell and Organ Culture. Minnesota: Burgess Publishing Co.; 1964.

40. Munson, R. J.; Goodhead, D. T. The relation between induced mutation frequency and cell survival--A theoretical approach and an examination of experimental data for eukaryotes. Mutation Research. 42:145-160; 1977.

41. Palmer, K. A.; Sheu, C. W.; Green, S. Mutagenicity studies of R-amino salt, a metabolite of amaranth (FD & C red no.2), in mouse lymphoma cells heterozygous at the thymidine kinase locus and in the rat dominant lethal test. Food and Cosmetics Toxicology. 17:5-9; 1979.

42. Penn, J. Procedure for testing impaction trapped WSCs. Notebook No. 7143, pp. 174-176.

43. Penn, J. M. Notebook No. 7288; pp. 31-54.

44. Penn, J. M. Notebook No. 7288; pp. 53, 54.

45. Penn, J. M. Notebook No. 7378, pp. 178-180.

46. Penn, J. M. L5178Y $TK^{+/-}$ assay--microsome evaluation: Aroclor® (Aro) XII *versus* X. Memo to R. A. Pages. 1978 August 9.

47. Penn, J. M. L5178Y $TK^{+/-}$ mouse lymphoma assay--WSC activities of the Model II cigarettes. Memo to R. A. Pages. 1978 December 6.

48. Rapp, K. E. Notebook No. 7015, p. 41.

49. Sanders, F. K. The effect of some nonviral oncogenic agents on mammalian cells *in vitro*. In: Nakahara, W.; Takayama, S.; Sugimura, T.; Odashima, S., eds. Topics in Chemical Carcinogenesis. Baltimore: University Park Press; 1972: 429-444.

50. de Serres, F. J.; Shelby, M. D.; eds. The International Program for the Evaluation of Short-term Tests for Carcinogenicity. An edited transcript of the public information meeting; Bethesda, MD. 1979 December 3.

51. Personal communication, D. Amacher.

52. Personal communication, D. Clive.

53. Personal communication, F. D. de Serres on the EPA's Office of Testing and Evaluation of the Gene-Tox Program.

54. Personal communication, V. C. Dunkel on the NCI four laboratory studies on the reproducibility of testing chemicals in short-term tests.

55. Personal communication from a talk presented by Dr. J. Spalding of NIEHS at the Second Annual Lymphoma Workshop, May 17-18, 1979 in Bethesda, MD.

56. Anonymous. Biotran II automated colony counter, Model C111, operating manual. New Brunswick Scientific Company, Inc.; New Jersey; 1976 July 16.

57. Anonymous. Cancer testing technology and saccharin. Office of Technology Assessment. Washington, DC: U. S. Government Printing Office; 1977: 49-82.

Legends to Tables and Figures

Table 1. The experiment was done as described in the MATERIALS AND METHODS section. It has been conducted several times using different treatment times and doses; the results were similar in each case. Variation from experiment to experiment is represented in Table 5.

Figure 1. Data obtained from Table 1; IMFs were plotted on the ordinate.

Table 2. The experiment was conducted as described in the MATERIALS AND METHODS section. The numbers were an average of at least three experiments.

Figure 2. Data obtained from Table 2; IMFs were plotted on the ordinate. Symbols are as follows: the circles (○, ●) are with microsomal activation and the squares (□, ■) are without microsomal activation.

Tables 3. The experiment was conducted as described in the MATERIALS AND METHODS section. The numbers were an average of at least three experiments.

Figure 3. Data obtained from Table 3; IMFs were plotted as the ordinate. See Figure 2 for the meaning of symbols.

Table 4. The experiment was conducted as described in the MATERIALS AND METHODS section. The numbers were an average of at least two experiments.

Figure 4. Data obtained from Table 4; IMFs were plotted on the ordinate. See Figure 2 for the meaning of symbols.

Table 5. These data were obtained from a summary of the ANOVAs across different classes for the control agents selected and the biological parameters measured (see Appendix D).

Table 6. EMS was not routinely included in every experiment that appeared in the data base of Appendix D and hence it could not be included in the evaluation that appears in Appendix D or in Table 5. Recently, accumulated data (February, 1979 to April, 1980) from 36 experiments were compiled for each assay parameter measured and the mean and standard deviation are presented here.

Table 1. Dose Response of EMS without Microsomal Activation

Column Numbers	2	3	4	5	6	7	8	9	10	11	12	13	14
	Expression Period Cell Counts x 10^6			Suspension Growth		Cloning Data							
Conc.	Day 1	Day 2	Day 3	Total[a]	%[b]	Viable Count x 10^6[c]	%[d]	$BUdR^Y$ x 10^2[e]	% Survival[f]	Mutation Frequency x 10^{-6}[g]	Log_e MF	x Spontaneous[h]	Induced Mutation Frequency x 10^{-6}[i]
Solvent Control	1.00	1.27	1.07	50.3	100	3.50	100	0.52	100	14.8	2.69	-	-
EMS 124 µg/ml	0.93	1.32	1.10	50.0	99.4	3.20	91.4	3.32	90.0	103.8	4.64	7.0	90
EMS 683 µg/ml	0.65	0.75	0.76	13.5	26.8	1.02	29.1	9.30	7.8	911.8	6.82	61.6	897
EMS 1242 µg/ml	0.39	0.43	0.33	2.05	4.1	0.09	2.6	2.35	0.1	2611.0	7.87	176.4	2596

---

[a] $\frac{\text{Day 1 cell count}}{0.3 \times 10^6} \times \frac{\text{Day 2 cell count}}{0.3 \times 10^{6}*} \times \frac{\text{Day 3 cell count}}{0.3 \times 10^{6}*}$

[b] $\frac{\text{total suspension growth of treated culture}}{\text{total suspension growth of control culture}} \times 100$

[c] $\frac{\text{average number of colonies/plate X dilution factor}}{20 \text{ ml}}$

[d] $\frac{\text{viable count of treated culture}}{\text{viable count of control}} \times 100$

[e] $\frac{\text{average number of colonies/plate X dilution factor}}{20 \text{ ml}}$

[f] $\frac{\text{\% suspension growth X \% viable count}}{100}$

* use previous day cell count if culture was not diluted

[g] $MF = \frac{BUdR^Y \times 10^2}{\text{viable count X } 10^6}$

[h] $\frac{\text{MF of treated culture}}{\text{spontaneous MF}}$

[i] IMF = MF - spontaneous MF

Figure 1. Dose Response of EMS

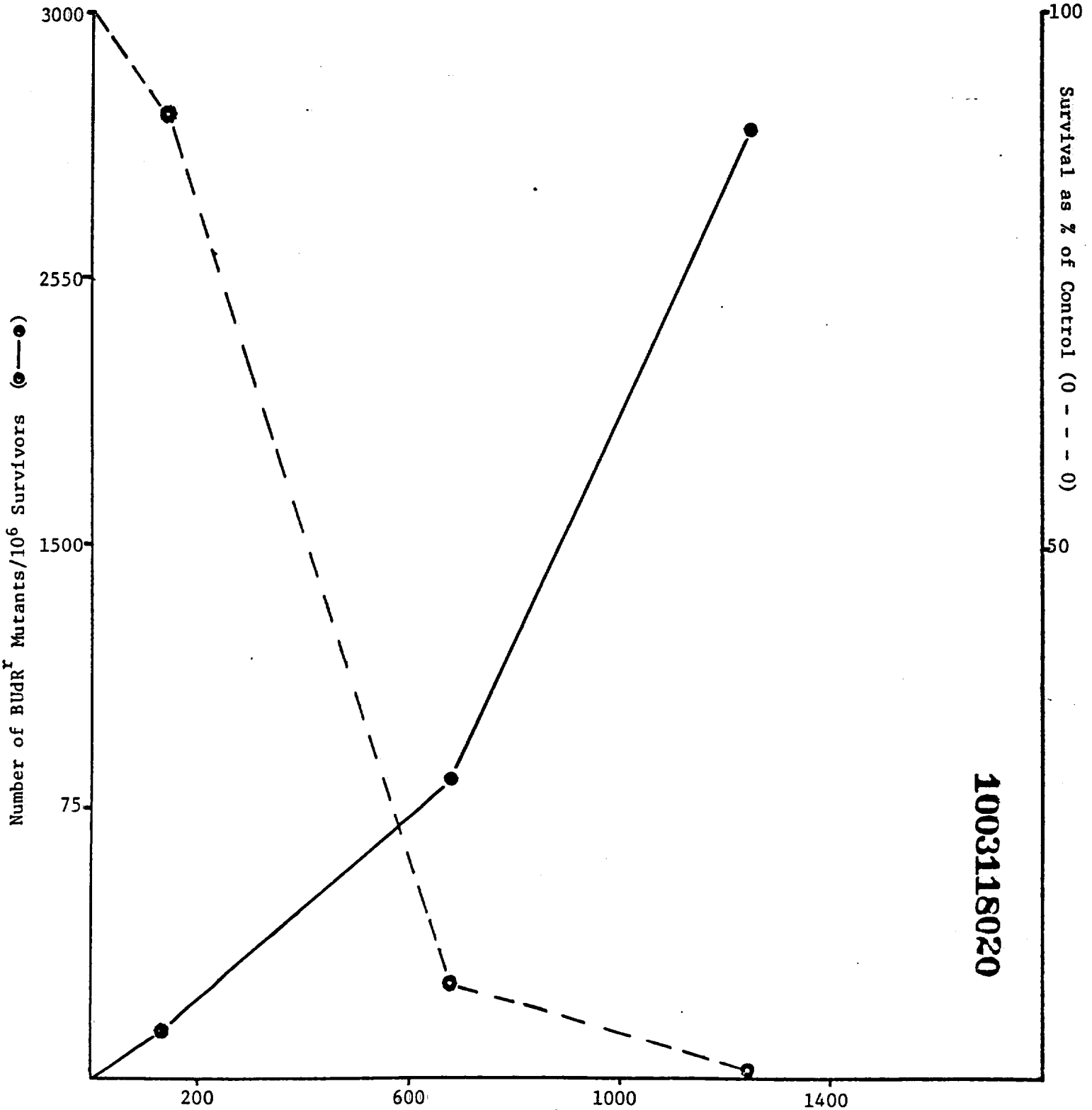


Table 2. Dose-Response of B(a)P with and without Microsomal Activation

S-9	Conc.	Expression Period X 10^6 D #1	D #2	D #3	Suspension Growth Total	%	Cloning Data Viable Count X 10^6	%	BUdR^r X 10^2	% Survival	Mutation Frequency X 10^{-6}	Log_e MF	X Spontaneous	Induced Mutation Frequency X 10^{-6}
yes	solvent control	1.26	1.18	1.44	79.3	100	2.84	100	0.77	100	27.1	3.30	-	--
yes	1 µg/ml	1.18	1.20	1.35	70.8	89.3	2.10	74	1.25	66	59.5	4.09	2.2	32
yes	3.5 µg/ml	1.10	1.14	1.37	63.6	80.2	1.63	57	2.38	46	146.0	4.98	5.4	119
no	solvent control	1.47	1.16	1.41	89.0	100	3.02	100	0.8	100	26.5	3.28	-	--
no	1 µg/ml	1.48	1.15	1.38	86.9	97.6	3.08	102	0.85	100	27.6	3.31	1.0	1
no	3.5 µg/ml	1.49	1.05	1.31	75.9	85.3	2.78	92	0.9	78	32.4	3.48	1.2	6

Figure 2. Dose Response of B(a)P with and without Microsomal Activation

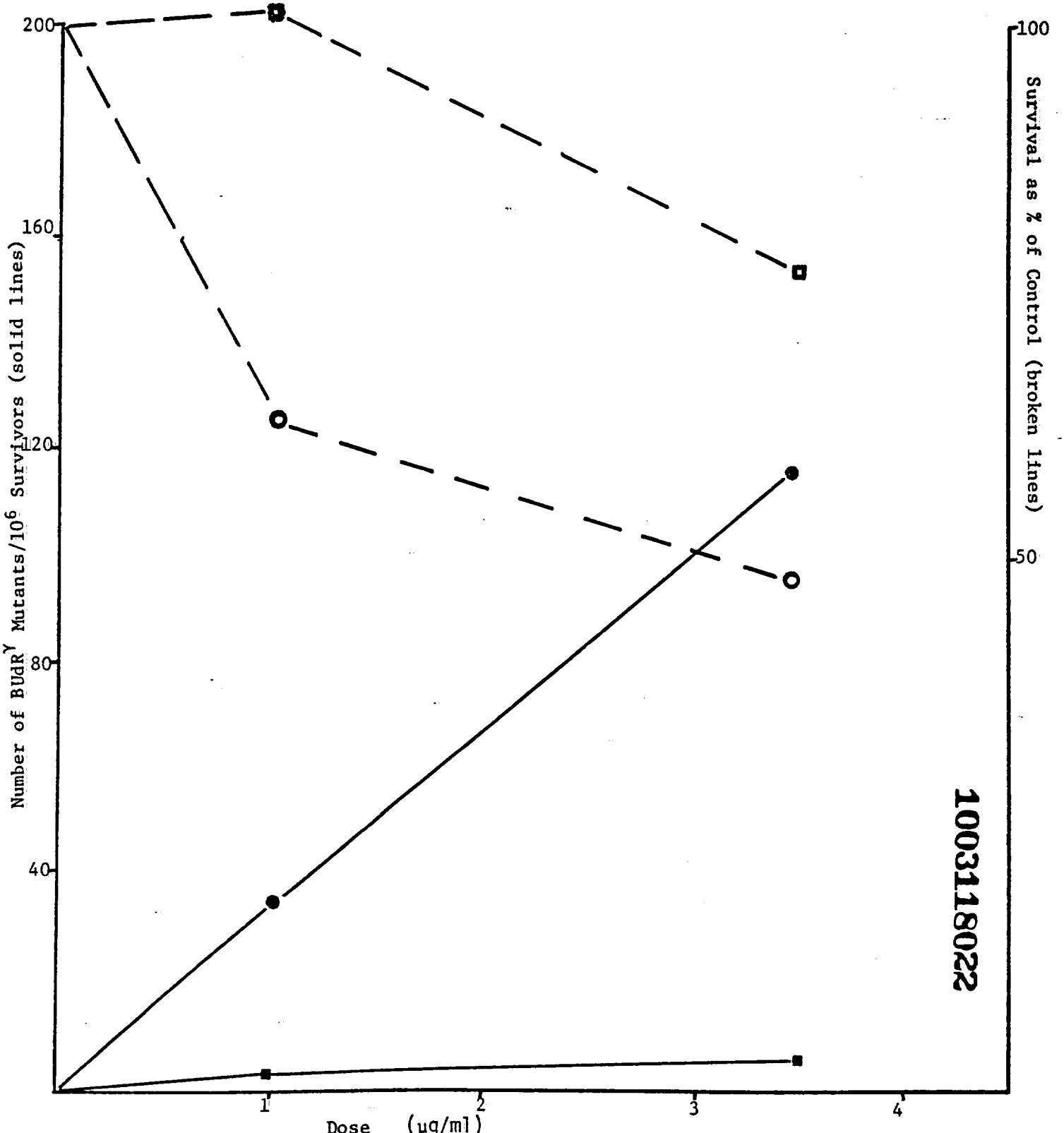


Table 3. Dose Response of 2-AAF with and without Microsomal Activation

S-9	Conc.	Expression Period X 10^6 D #1	D #2	D #3	Suspension Growth Total	%	Cloning Data Viable Count X 10^6	%	BUdRr X 10^2	% Survival	Mutation Frequency X 10^{-6}	Log_e MF	X Spontaneous	Induced Mutation Frequency X 10^{-6}
yes	solvent control	1.41	1.31	1.38	94.4	100	2.4	100	0.63	100	26.0	3.26	--	--
yes	25 µg/ml	0.94	1.21	1.39	58.6	62.1	2.36	98.3	1.06	61.0	44.9	3.80	1.7	19
yes	50 µg/ml	0.55	0.95	1.21	23.4	24.8	1.75	72.9	1.96	18.1	112.1	4.72	4.3	86
yes	75 µg/ml	0.68	0.99	1.22	30.4	32.2	1.01	42.1	2.13	13.6	209.9	5.35	8.1	184
no	solvent control	1.62	1.17	1.52	107.4	100	2.4	100	0.67	100	27.9	3.32	--	--
no	25 µg/ml	1.50	1.22	1.37	92.9	98.4	2.1	87.5	0.73	86.1	34.5	3.54	1.2	7
no	50 µg/ml	1.38	1.18	1.37	82.6	87.5	2.7	112.5	1.3	98.4	48.1	3.87	1.7	20
no	75 µg/ml	1.34	1.16	1.43	82.3	87.2	1.54	64.2	1.29	56.0	83.7	4.43	3.0	56

Figure 3. Dose Response of 2-AAF with and without Microsomal Activation

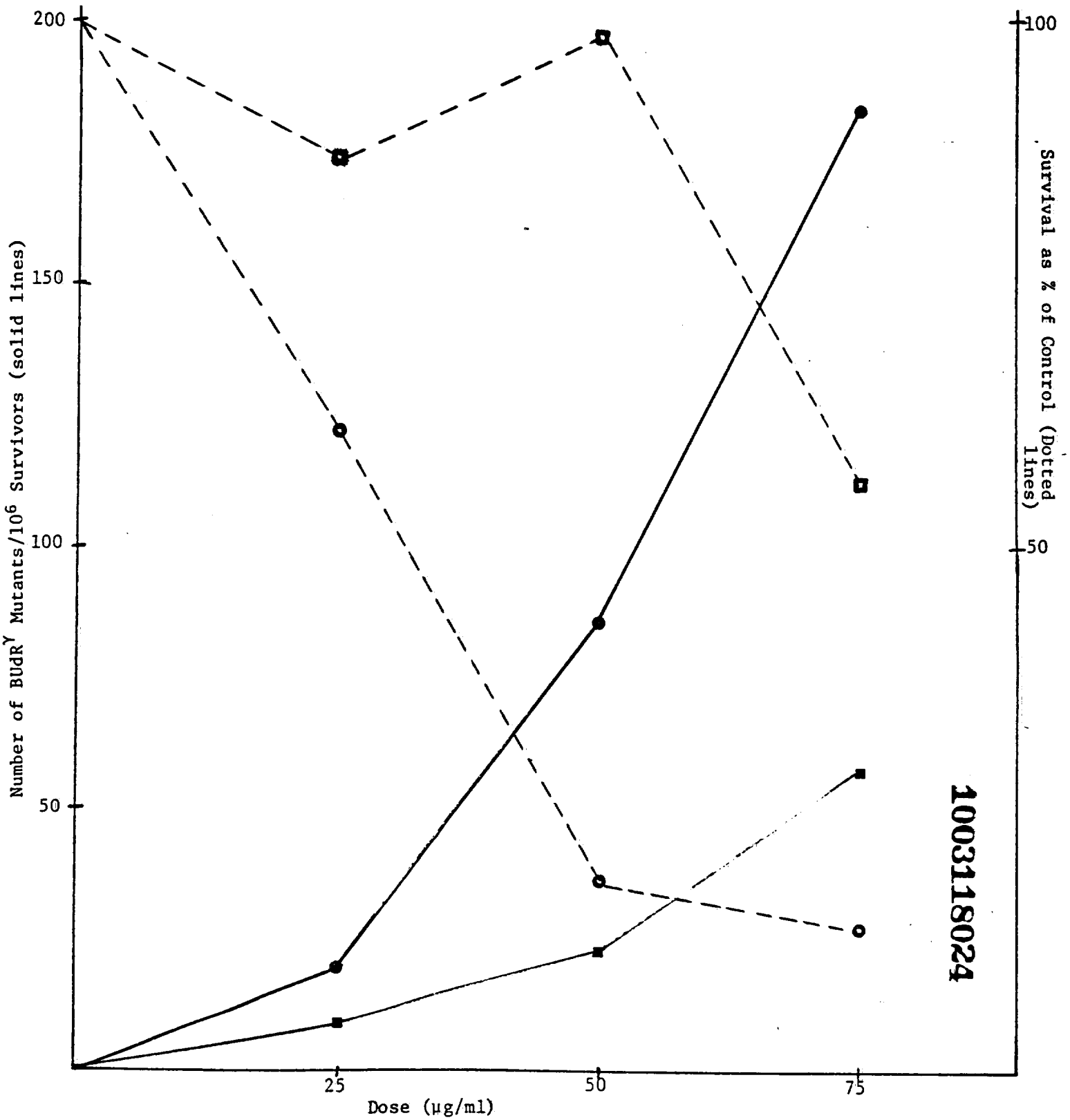


Table 4. Dose-Response of 2R1 WSC with and without Microsomal Activation

S-9	Conc.	Expression Period X 10^6 D #1	D #2	D #3	Suspension Growth Total	%	Cloning Data Viable Count X 10^6	%	BUdR^r X 10^2	% Survival	Mutation Frequency X 10^{-6}	Log_e MF	X Spontaneous	Induced Mutation Frequency X 10^{-6}
yes	solvent control	1.14	1.11	1.42	66.6	100	3.70	100	1.60	100	43.2	3.77	--	--
yes	50 μg/ml	1.07	1.12	1.38	61.3	92.0	2.15	58	0.95	53.4	44.2	3.79	1.0	1
yes	100 μg/ml	0.83	1.03	1.42	45.0	67.6	2.31	62	1.81	41.9	78.4	4.36	1.8	35
yes	200 μg/ml	0.44	0.94	1.30	19.0	29.9	1.32	36	2.55	10.8	193.2	5.26	4.5	150
yes	400 μg/ml	0.21	0.08	0.01	culture dying; not cloned→									
no	solvent control	0.98	1.0	1.38	50.1	100	2.10	100	1.05	100	50	3.91	--	--
no	50 μg/ml	0.42	0.90	1.18	16.5	32.9	2.15	102	2.00	33.6	93	4.53	1.9	43
no	100 μg/ml	0.25	0.06	culture dying; not cloned→										
no	200 μg/ml	0.18	0.04	culture dying; not cloned→										
no	400 μg/ml	0.20	0.02	culture dying; not cloned→										

Figure 4. Dose Response of 2R1 WSC with and without Microsomal Activation

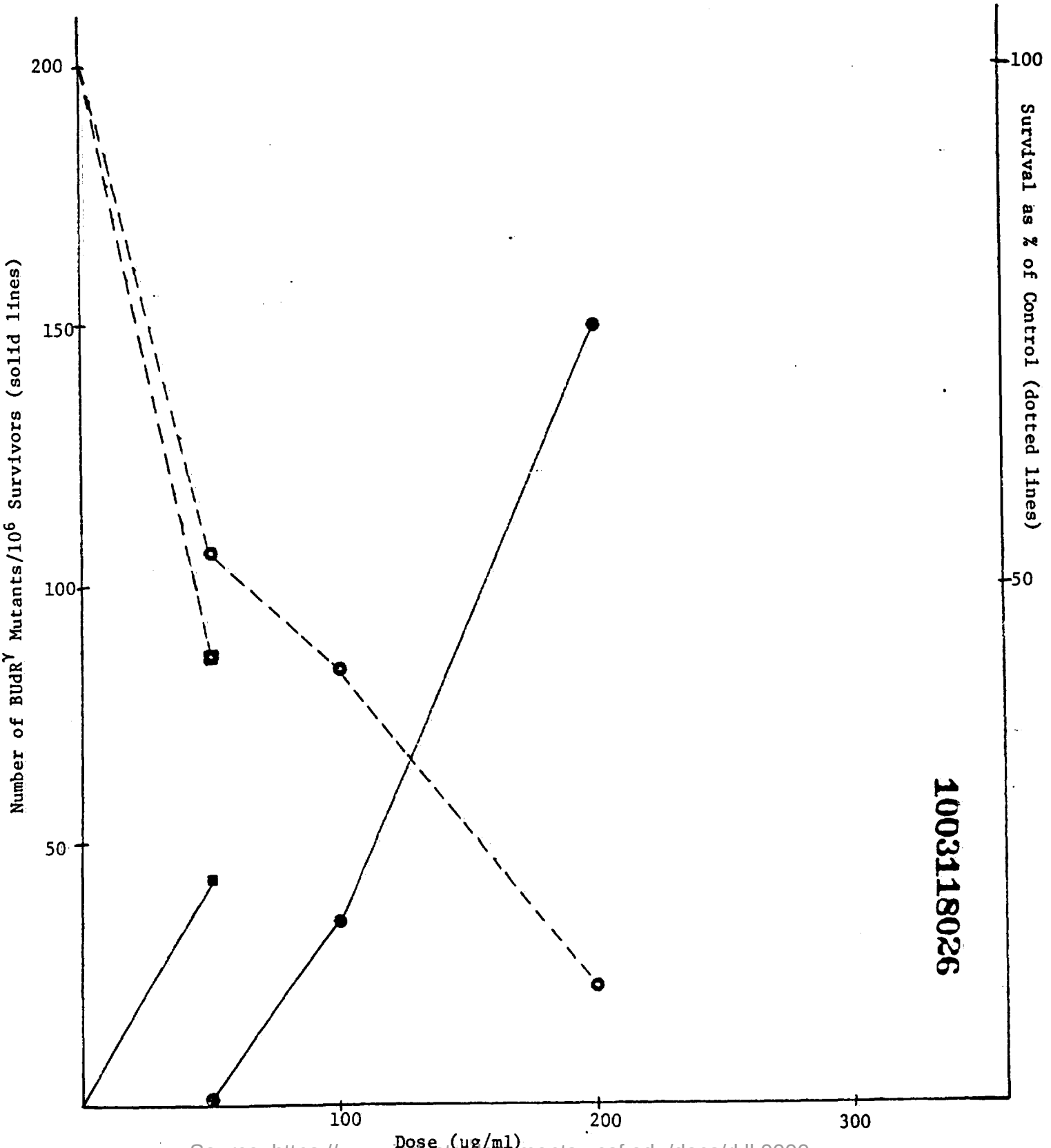


Table 5. Summary of the Analyses from Appendix D

	Total Suspension Growth[a]	Percent Cloning Efficiency[a]	Percent Survival[a]	Mutation Frequency[a]	$\log_e$ Mutation Frequency[a]
DMSO	80 ± 28	79 ± 26	100[b]	32.5 ± 23	3.4 ± 0.67
B(a)P	58 ± 28	67 ± 23	62 ± 24	159 ± 69	5 ± 0.4
2-AAF	29.5 ± 11	57 ± 15	26 ± 8	154 ± 11	5.0 ± 0.5
2R1	8.8 ± 17	61 ± 18	8 ± 13	149 ± 88	5 ± 1

[a]Mean ± standard deviation

[b]This number is arbitrarily set at 100%.

Table 6. Nonactivation Parameters[a]

	Total Suspension Growth	Percent Cloning Efficiency	Percent Survival	Mutation Frequency
DMSO[b]	92 ± 17[c]	78 ± 17	100[d]	36 ± 9
EMS[e]	27 ± 8	38 ± 12	14 ± 5	1076 ± 278

[a]These values were compiled from experiments completed between 2/79 and 8/20/80 and were not analyzed in Appendix D or included in Table 5.

[b]These values represent 31 different experiments.

[c]Mean ± standard deviation.

[d]This is arbitrarily set at 100%.

[e]These values were calculated from 41 experiments.

APPENDIX A

PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: . Distribution

Date: September 28, 1979

From: . W. R. McCoy

Subject: . Procedure for Handling 30-gallon Metal Drums for Disposal of Solid Hazardous Waste Material

In the fall of 1977, the Research Center contracted RAD Services, Inc. to dispose of hazardous chemical waste.[1] Part of this service involves the handling, by PM personnel, of 30-gallon metal drums. On March 15, 1978, a meeting was held with members of Charge Number 6906 and the stockroom personnel of the Administrative Services Division to clarify the logistics of handling waste disposal drums.[2] The meeting resulted in a protocol being proposed and accepted. Since that time, some of the handling procedures have been changed, discontinued, or otherwise modified. The purpose of this document is to update and further clarify the procedures and responsibilities for handling 30-gallon metal drums for disposal of hazardous waste material. The procedures and responsibilities were reviewed and agreed upon in consultation with all of the personnel affected by this protocol.

The current protocol is as follows:

1. RAD Services, Inc. will provide PM with 30-gallon drums to which vermiculite has been added to a depth of 3-4 inches. Also provided are top-sealing gaskets and nuts and bolts for securing tops to the drums. The R & D stockroom (Mr. Mark Davis or his designee) will provide plastic bags for the covering of full drums.

2. Empty drums, along with accessories described above, will be transferred from the stockroom area to the Tower storage area (outside storage on the north side of the Tower) by stockroom personnel. These materials will be stored in the Tower storage area for exclusive use by the members of Charge Numbers 6906 and 6908. In order to provide protection of accessories described above from the weather, a lidded plastic trash container is located in the storage area for these items.

3. A short ramp constructed of heavy gauge aluminum has been provided so that a cart can be moved in and out of the storage area. A laboratory cart, which will be used to transport the empty as well as the full drums, has been provided to 6906 and 6908 personnel. This cart is normally kept in the T-6 north chase when not in use.

4. The door leading into the designated storage area will be padlocked. Keys to this lock will be retained by Mr. Davis, Mr. W. R. McCoy (who has been designated as having primary responsibility for coordination of toxic waste disposal operations in the Biochemical Research Division), and Mrs. Nancy Peters, Biochemical Research Division Secretary.

5. Members of Charge Numbers 6906 and 6908 will be responsible for the transportation of drums between their respective laboratories and the Tower storage area (for further details, see #9 below).

6. Once an empty drum is placed in the laboratory, it may remain there until it is filled. [However, as each drum is put into use, it will be marked "Caution Cancer Suspect Agent" and/or "Cancer Suspect Agent. Authorized Personnel Only." These self-adhesive, 6 x 11.5 cm labels are available from Mr. McCoy (T613).] Drums should be filled with solid waste only (petri dishes, pipettes, test tubes, *etc.*). Liquid waste should not be placed in these drums. This is in compliance with Department of Transportation (DOT) regulations. A detailed procedure for the handling of liquid hazardous waste material is in preparation.

7. Once a drum has been filled, the top, with sealing gasket in place, should be firmly secured. (The 6906/6908 personnel responsible for filling the drum are also responsible for sealing it.) Care should be taken to place the fastening bracket with nut and bolt in the "down" position (*i. e.*, below the top surface of the drum). Tools to assist in the performance of this operation are located in room T-613 (or see Mr. McCoy).

8. Members of Charge Numbers 6906 and 6908 will be responsible for the transportation of these full drums between their respective laboratories and the Tower storage area. The drum will be strapped to the laboratory cart (see paragraph 3 above) and transported by two people to the storage area. Transportation of drums on the Tower freight elevator will be accomplished with the drum movers having sole control of the elevator. This is done through the use of an elevator key, which will be retained by Mrs. Nancy Peters.

9. Upon exit through the Tower receiving door to the drum storage area, the receiving door must be closed. This is accomplished by arranging for mail room personnel to close the door after exit. To reenter the building, a signal bell, located adjacent to the outside of the receiving door, is pressed. Mail room personnel will then reopen the door.

10. Once a full, labeled (see #6 above) drum has been placed in the designated storage area, it must be covered with a plastic bag. The bag will also be marked, "Caution Cancer Suspect Agent." This will no longer be required upon the installation of a roof over the storage area.

11. Mr. Davis is responsible for transporting the full drums from the designated Tower storage area to the stockroom receiving area for pickup by RAD Services personnel. Before these full drums are loaded onto the RAD Services truck, representatives of RAD Services, Inc. will mark these drums with a label, "Biological Agent." (They provide the label which serves to comply with government regulations concerning the currently acceptable method for transporting and disposal of such waste materials.)

12. Mr. Davis, only, will inform RAD Services, Inc. to arrange their schedule for a pickup of the full drums and/or the delivery of empty drums as necessary.

References:

1. Thomson, R. N. Hazardous material waste disposal. Memo to Managers; 1977 October 27.

2. Kuhn, W. F. Waste disposal for Charge Number 6906. Memo to distribution; 1978 March 22.

nwp

W. R. McCoy

Distribution:

Dr. T. S. Osdene
Mr. R. N. Thomson
Mr. W. F. Kuhn
Mr. J. L. Charles
Dr. R. A. Pages
Dr. R. N. Ferguson
Ms. R. D. Kinser
Mr. J. J. Cacciotti
Mr. B. J. Kosakowski
Mr. R. E. Tinker
Mr. K. R. Newman
Mr. M. Davis

PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: . Distribution Date: August 28, 1980

From: . W. R. McCoy

Subject: . Revised Procedure for Handling 30-Gallon Metal Drums for Disposal of Solid Hazardous Waste Material.

A memo has previously been issued concerning the procedure for the handling of 30-gallon metal drums for the disposal of solid hazardous waste material generated or used by some members of the Biochemical Research Division.[1] A more recent memo reported an incident, and questions raised by that incident, involving this procedure.[2]

The two major issues, of which this memo is designed to resolve, are as follows. Mr. Barbee (Accounts Manager of Triangle Resource Industries - TRI) informed us that, due to the recent expansion of hazardous waste regulations by EPA under the authority of the Resource Conservation and Recovery Act (RCRA)[3], we have to put in writing what hazardous substances are in each drum along with an estimate of their concentrations. This information will have to be attached to each drum. The second issue, pointed out by Mr. Barbee, is that the contents of each drum, to which viable organisms are added, must be treated with formalin (37% formaldehyde in water) in order to satisfy the regulations of the disposal site (Solid Waste Management - Livingston, Alabama) utilized by TRI.

In order to comply with the above two requests, the procedure for handling 30-gallon metal drums for disposal of solid hazardous waste material is modified as follows. To each waste drum will be taped a plastic pocket (8½" x 12" size which is available from the PM mailroom) for holding PM Hazardous Waste Forms (available from W. McCoy). Attached to this memo is an example of the PM Hazardous Waste Form correctly filled out to comply with TRI and Federal regulations.

Before any materials are added to the drum, the inside of the drum must be lined with a large plastic bag (available from the R&D stockroom). Then, as materials are added to the drum, they must be noted on the PM Hazardous Waste Form along with the disposer's name, date, etc. (see attachment). Once the drum is filled, 500 ml of formalin (37% formaldehyde available from the R&D stockroom) is poured over the drum contents into the plastic drum liner. Since formalin itself is hazardous, this operation should be conducted with caution. (Gloves and lab coat should be worn, and drum should be in or adjacent to a fume hood). After the formalin has been added, the plastic bag is secured (this may be accomplished with a rubber band or string). The drum top, with sealing gasket in place, is then firmly attached. The PM Hazardous Waste Form is now completed by having both the project leader and the manager sign and date the form. Two copies of the completed Waste Form are placed in the plastic pocket taped to the drum. After the filled and formalin treated drum has remained in the laboratory for 24 hours, it is then transported to the Tower storage area. The plastic drum liner and formalin treatment procedure may be omitted for drums to which no viable organisms are added.

Other minor changes have been made in the earlier procedure[1] for handling 30-gallon metal drums for disposal of solid hazardous waste material. You will find, attached, the revised protocol in step-by-step outline form with details of how the procedure is to now be followed.

References

1. McCoy, W. R., Procedure for Handling 30-Gallon Metal Drums for Disposal of Solid Hazardous Waste Material. Memo to distribution; 1979 Sept. 28.

2. McCoy, W. R. and McCuen, R., Additional Requirements for the Disposal of Hazardous Wastes. Memo to distribution; 1980 June 25.

3. Anonymous. Environmental Protection Agency, Hazardous Waste and Consolidated Permit Regulations. Federal Register. 45 (98):33063-33285. 1980 May 19. (40 CFR part 260).

Distribution:

Dr. T. S. Osdene
Mr. R. N. Thomson
Mr. W. F. Kuhn
Mr. J. L. Charles
Dr. R. A. Pages
Dr. R. N. Ferguson
Ms. R. D. Kinser
Mr. J. J. Cacciotti
Mr. B. J. Kosakowski
Mr. K. R. Newman
Mr. M. Davis

W. R. McCoy

WRM/atd

ATTACHMENT 1

Revised Procedure for Handling 30-Gallon Metal Drums for Disposal of Solid Hazardous Waste Material

The revised protocol is as follows:

1. TRI will provide PM with 30-gallon drums to which vermiculite has been added to a depth of 3-4 inches. Also provided are top-sealing gaskets and nuts and bolts for securing tops to the drums. The R & D stockroom (Mr. Mark Davis or his designee) will provide plastic bags for the lining of drums.

2. Empty drums, along with accessories described above, will be transferred from the stockroom area to the Tower storage area (outside storage on the north side of the Tower) by stockroom personnel. These materials will be stored in the Tower storage area for exclusive use by the members of Charge Numbers 6906 and 6908.

3. A short ramp constructed of heavy gauge aluminum has been provided so that a cart can be moved in and out of the storage area. A laboratory cart, which will be used to transport the empty as well as the full drums, has been provided to 6906 and 6908 personnel. This cart is normally kept in the T-6 north chase when not in use.

4. The door leading into the designated storage area will be padlocked. Keys to this lock will be retained by Mr. Davis, Mr. W. R. McCoy (who has been designated as having primary responsibility for coordination of toxic waste disposal operations in the Biochemical Research Division), and Mrs. Nancy Peters, Biochemical Research Division Secretary.

5. Members of Charge Numbers 6906 and 6908 will be responsible for the transportation of drums between their respective laboratories and the Tower storage area (for further details, see #9 below).

6. Once an empty drum is placed in the laboratory, it may remain there until it is filled. However, as each drum is put into use, it will be marked "Caution Cancer Suspect Agent" and/or "Cancer Suspect Agent: Authorized Personnel Only." These self-adhesive, 6 x 11.5 cm labels are available from Mr. McCoy (T-712). Also, to each drum will be taped a plastic pocket (8½" x 12" size which is available from the PM mailroom) for holding the PM Hazardous Waste Forms (available from W. McCoy).

Attached to this memo is an example of the PM Hazardous Waste Form correctly filled out according to TRI and

Federal regulations. Before any materials are added to the drum, the inside of the drum must be lined with a large plastic bag (available from the R & D stockroom). Then, as materials are added to the drum, they must be noted on the PM Hazardous Waste Form along with the disposer's name, date, etc. (see attachment). Drums should be filled with solid waste only (petri dishes, pipettes, test tubes, etc.). Liquid waste should not be placed in these drums. A revised procedure for the handling of liquid hazardous waste material is in preparation.

7. Once the drum is filled, 500 ml of formalin (37% formaldehyde, available from the R & D stockroom) is poured over the drum contents into the plastic drum liner. (This formalin treatment procedure may be omitted for drums to which no viable organisms are added). Since formalin itself is hazardous, this operation should be conducted with caution. (Gloves and lab coat should be worn, and drum should be in or adjacent to a fume hood). After the formalin has been added, the plastic bag (liner) is secured (this may be accomplished with a rubber band or string).

8. The drum top, with sealing gasket in place, is firmly secured. (The 6906/6908 personnel responsible for filling the drum are also responsible for sealing it.) Care should be taken to place the fastening bracket with nut and bolt in the "down" position (i.e, below the top surface of the drum). Tools to assist in the performance of this operation are located in room T-712 (or see Mr. McCoy).

9. The PM Hazardous Waste Form is now completed by having both the project leader and the manager sign and date the form. Two copies of the completed Waste Form are placed in the plastic pocket taped to the drum.

10. After the formalin treated drum has remained in the laboratory for 24 hours, it is then transported to the Tower storage area.

    Members of Charge Numbers 6906 and 6908 will be responsible for the transportation of these full drums between their respective laboratories and the Tower storage area. The drum will be strapped to the laboratory cart (see paragraph 3 above) and transported by two people to the storage area. Transportation of drums on the Tower freight elevator will be accomplished with the drum movers having sole control of the elevator. This is done through the use of an elevator key, which will be retained by Mrs. Nancy Peters.

11. Upon exit through the Tower receiving door to the drum storage area, the receiving door must be closed. This is accomplished by arranging for mail room personnel to close the door after exit. To reenter the building, a signal

bell, located adjacent to the outside of the receiving door, is pressed. Mail room personnel will then reopen the door.

12. The PM Hazardous Waste Form must remain attached to the metal drum via the plastic pocket to aid Mark Davis and TRI personnel in loading the drums for interstate shipment and burial in Alabama.

13. Mr. Davis, only, will inform TRI to arrange their schedule for a pickup of the full drums and/or the delivery of empty drums as necessary.

ATTACHMENT 2

PM HAZARDOUS WASTE FORM

Item	Chemical Name of Waste*	Amount Discarded (Weight and/or Volume)	Generator and Date
Lab coat		One	S. Drew
Gloves		One pair	S. Drew
Lab coat		One	W. McCoy
Gloves		One pair	W. McCoy
Test tubes containing the following:	Dimethyl sulfoxide	< 1 µg	S. Drew
	Nicotine and nicotine salts	< 1 µg	"
	2-aminoanthracene	< 1 µg	"
	2-nitrofluorene	< 1 µg	"
	Sodium azide	< 1 µg	"
Petri Plates containing the following	Nicotine and nicotine salts	200 mg	W. McCoy
	Dimethyl sulfoxide	100 ml	"
	2-aminoanthracene	2 µg	"
	2-nitrofluorene	13 µg	"
	Sodium azide	3 µg	"
	Formalin (37% formaldehyde)	500 ml	"

* No abbreviations; list complete name of all chemicals

GENERATED BY
Building/Room Number - Tower/712
Responsible Person - (Project Leader)
Date -

I certify that the above named materials are properly classified and described according to applicable regulations of the DOT.

(Manager)
name and title - typed

Date

Signature

1003118038

APPENDIX B

PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Distribution

Date: November 16, 1979

From: R. W. McCuen

Subject: Procedure for Handling the Disposal of Hazardous Liquid Waste Material

In the fall of 1977, the Research Center contracted RAD Services, Inc. to dispose of hazardous chemical waste.[1] Part of this service involves the handling, by PM personnel, of 30-gallon metal drums which are used for the disposal of hazardous solid waste material. An initial protocol[2] concerning the disposal of solid waste material has recently been updated,[3,4] circulated to interested personnel, and adopted for use by members of the Biochemical Research Division.

The purpose of this document is to outline the procedures to be used by personnel of the Biochemical Research Division for the handling of hazardous liquid waste materials so as to make the task of liquid waste removal safer for all of us.

The current protocol, which should be adhered to by all members of the Biochemical Research Division, is as follows:

1. What is a hazardous liquid waste material?

Any liquid which exposes an employee to risk! In many cases the risk is unknown at which time the *potential* risk should be considered. *Some* examples of hazardous or potentially hazardous liquid chemical wastes are carcinogens, mutagens, toxins, and other such biologically active agents (genotoxic substances) or solutions containing such materials. Washings from Elmenhorst and impaction traps, materials stripped during condensate or TPM processing, or concentration of WSC fractions should be considered waste.

2. All hazardous liquid wastes will be stored in one-gallon solvent bottles which are contained in Saftepak™ metal cans. [These containers, which hold eight pints, may be purchased from Matheson, Coleman and Bell of Norwood, OH for $8.75 each. The metal container encloses a glass bottle (noncorrosive) which is surrounded by corrugated cardboard and covered with a protective plastic cap over the glass bottle cap. Tests conducted by our laboratory personnel using Saftepaks™ containing one-gallon of water showed that they can be dropped from a height of about two feet onto the floor without breaking the inner bottle. However, if the filled container was dropped on its side under the same conditions, the glass bottle was shattered and leakage occurred at the screw cap of the metal container.]

3. In the laboratory, each in use Saftepak™ (having been pretested for leaks by filling it with water) will be stored in a suitable container (*e. g.*, metal tray capable of holding one gallon of liquid) in an effort to keep the liquid contents confined to a relatively small area should a break in the bottle and a leak in the protective can occur. The storage area in each laboratory will be up to the individuals within the laboratory, however, it is advised that the storage area(s) be located in lightly traveled or little used locations. In addition, it is recommended that the storage area(s) not be located near sources of heat (autoclaves, *etc.*) or near equipment with fans or blowers on them. Such storage areas may tend to volatilize and/or disperse hazardous chemical vapors into the laboratory.

4. Each in use Saftepak™ container will be marked "Caution Cancer Suspect Agent, Authorized Personnel Only." These self-adhesive, 6 x 11.5 cm labels are available from Lab Safety Equipment and Supplies, 3430 Palmer Drive, Jonesville, WI 53545, catalogue number B173K or Mr. W. R. McCoy.

5. To each Saftepak™ container will be taped a plastic pocket (8 1/2" x 12" size which is available from the PM mailroom) for holding the chemical disposal request sheet (available from the PM stockroom). Attached to this memo is an example of the chemical disposal request sheet and how to *correctly fill it out* according to RAD and the Department of Transportation regulations. As chemical solutions are added to the container, they must be noted on the disposal request sheet. The burden of safely adding chemicals to the Saftepak™ container is up to each individual using this system. Do not jeopardize your safety and/or that of your co-workers by improper use of these containers. If you are unsure of what chemical reactions may occur as a consequence of what you are adding to the container, ask your Project Leader before you add!!

6. When the Saftepak™ container is full, the white copy of the chemical request sheet is sent to the PM stockroom (care of Mr. M. Davis). The pink copy of the request sheet must remain attached to the metal container via the plastic pocket to aid RAD personnel in packing the Saftepaks™ into 30-gallon, open head drums for interstate shipment and burial in Alabama.

7. When Mr. Davis determines, that from the chemical disposal request sheets, PM has enough liquid waste materials (including the containers) to fill at least one, 30-gallon drum, he calls RAD to have them pick up the material as soon as possible.

8. In the morning of the day RAD is to come to PM for the pickup, or if possible, prior to the pickup day, Mr. Davis, using the chemical disposal requests sheets, notifies the sender of the sheet to bring his or her waste chemicals to the stockroom.

9. The transportation of the filled Saftepak™ containers from the lab to the stockroom will be accomplished by placing the container in a rubber reagent bottle carrier (available from the stockroom). This will virtually eliminate any serious spills from occurring between the lab storage area and the stockroom. It is suggested that transportation from the lab to the stockroom be done at times when large crowds are not likely to be in the halls; *e. g.*, at times other than during lunch or morning and afternoon break periods. Should a spill occur this will minimize exposure to other PM personnel.

10. The filled Saftepaks™ should be stored in the stockroom as safely as possible (see item number 3 of above) until their removal by RAD personnel can be accomplished.

11. It should be noted that not all hazardous liquid wastes must be stored in Saftepak™ containers. They may be stored in small glass vials or tubes as long as they are sealed with a screw cap top. For disposal, these containers may be placed in a plastic bag or large Mason jar suitably labeled with a chemical disposal request sheet. They may be transferred to the stockroom in any safe manner (metal tray, rubber reagent carrier, and see item number 9 of above) as determined by PM personnel.

References:

1. Thomson, R. N. Hazardous material waste disposal. Memo to Managers; 1977 October 27.
2. Kuhn, W. F. Waste disposal for Charge Number 6906. Memo to distribution; 1978 March 22.
3. Kuhn, W. F. Protocol for disposal of toxic wastes. Memo to distribution; 1979 August 9.
4. McCoy, W. R. Procedure for handling 30-gallon metal drums for disposal of solid hazardous waste material. Memo to distribution; 1979 September 28.
5. Kuhn, W. F. Protocol for disposal of hazardous liquid waste material. Memo to distribution; 1979 September 26.

np

Distribution:
Dr. T. S. Osdene
Mr. R. N. Thomson
Mr. W. F. Kuhn
Mr. J. L. Charles
Dr. R. A. Pages
Dr. R. N. Ferguson
Ms. R. D. Kinser
Mr. J. J. Cacciotti
Mr. B. J. Kosakowski
Mr. R. E. Tinker
Mr. K. R. Newman
Mr. M. Davis

Attachment

CHEMICAL DISPOSAL REQUEST [1]

NAME OF CHEMICAL(s) [2]	QUANTITY [3]	TYPE [4]	HAZARD CLASS [5]
2-acetylaminofluorene	5 ml dimethyl sulfoxide at 5 mg/ml	non-flammable	
ethyl methanesulfonate	2 ml concentrated or undiluted material	non-flammable	
injection trap flask washings	350 ml acetone	flammable	
unknown mixture	100 ml dimethylsulfoxide	non-flammable	

1 This sheet was originally prepared by Mr. R. Newman of SM as a general answer to R&D's waste disposal problems. For the Biochemical Research Division's needs, the sheet has been modified as you see below.

2 Full name (not abbreviation) of chemical(s) where known.

NAME OF REQUESTOR L. McCuen

DATE REQUESTED [6] 11/12/79

PHONE EXTENSION 5840

Return completed forms to the Stockroom. Notification will be made prior to date of pick up.

3 R&D must know (where possible) the concentration of the chemical as well as the solvent that it's in.

4 This refers to the type of chemical one is discarding such as an oxidant, flammable, non-flammable, etc. R&D at this time is interested only in the flammability of the discarded chemical or solvent.

5 This designation is unnecessary since all of the Biochemical Research Division's liquid wastes are treated as hazardous.

6 This may be filled out either as the lab you with the waste is removed from the lab or the date which the top sheet of the disposal request is sent to the stockroom (gives one a record of the elapsed time between notification of disposal and the actual disposal).

100311804 3

PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Distribution

Date: December 18, 1979

From: R. W. McCuen

Subject: Addendum to the Procedure for Handling the Disposal of Hazardous Liquid Waste Material

As indicated in the Biochemical Research Division's November Safety Meeting, implementation of the Liquid Waste Disposal Procedure[1] could not begin until we received the Saftepak® containers from Matheson, Coleman, and Bell. Even though the Research Center obtains several kinds of solvents (acetone, methanol, *etc.*) from MCB in these containers, I understood that reusing the empty containers was not allowed. I have recently been informed (J. Cacciotti, personal communication) that this is not true.

In an effort to save time and money (new or unused Saftepaks® cost $8.75 each plus paperwork and handling), we will use the empty, glass bottle-containing Saftepaks® generated by personnel within the Biochemical Research Division. As many as a dozen or more containers per month may be obtained from Ralph Hellams of Charge Number 6908. The PM Stockroom's supply of new Saftepaks® will act as a backup source of containers should they be needed in the future.

Because Saftepak® containers are now available, implementation of the Liquid Waste Disposal Procedure can begin without further delay.

---

[1]McCuen, R. W. Procedure for handling the disposal of hazardous liquid waste material. Memo to distribution; 1979 November 16.

nwp

R. McCuen

Distribution:
Dr. T. S. Osdene
Mr. R. N. Thomson
Mr. W. F. Kuhn
Mr. J. L. Charles
Dr. R. A. Pages
Dr. R. N. Ferguson
Ms. R. D. Kinser
Mr. J. J. Cacciotti
Mr. B. J. Kosakowski
Mr. R. E. Tinker
Mr. K. R. Newman
Mr. M. Davis
Biochemical Research Division

1003118044

PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: . Distribution

Date: September 3, 1980

From: . R. W. McCuen

Subject: . Addendum to the Procedure for Handling the Disposal of Hazardous Liquid Waste Material.

Recent conversations with the Accounts Manager of Triangle Resource Industries (TRI, formerly RAD Services, Inc.)[1] concerning the proper labeling of the hazardous liquid waste containers has led to the following modification of the Procedure for Handling the Disposal of Hazardous Liquid Waste Material.[2] Item number 5 of the Procedure deals with completing the chemical disposal request sheet according to TRI and DOT (Department of Transportation) regulations. These regulations have been superseded by the Environmental Protection Agency's (EPA) labeling requirements. Therefore, effective immediately, all hazardous liquid waste containers must have attached to the container an information form which embodies all the requirements of the EPA's Hazardous Waste and Consolidated Permit Regulations.[3] A copy of this form, correctly filled out, is attached to this memo. Each time an addition is made to the liquid waste container, the form must be updated. Please note that the above information also applies to item number 11 of reference 2. When containers are transported to the stockroom, a Xeroxed copy of the completed form (signed) must be made. Thus, 2 copies of the waste disposal form must be attached to the container to be discarded. One form remains with the container, the other is retained by PM personnel. Blank forms are available from the author.

The remaining items as stated in the Procedure for Handling the Disposal of Hazardous Liquid Waste Material[2] remain unchanged.

RWM/atd

1003118045

References

1 McCoy, W.; McCuen, R. Additional requirements for the disposal of hazardous wastes. Memo to distribution; 1980 June 25.

2 McCuen, R. W. Procedure for handling the disposal of hazardous liquid waste material. Memo to distribution; 1979 November 16.

3 Anonymous; Environmental Protection Agency, Hazardous Waste and Consolidated Permit Regulations. Federal Register. 45(98):33063-33285; 1980 May 19.

Distribution:

Dr. T. S. Osdene
Mr. R. N. Thomson
Mr. W. F. Kuhn
Mr. J. L. Charles
Dr. R. A. Pages
Dr. R. N. Ferguson
Ms. R. D. Kinser
Mr. J. J. Cacciotti (2)
Mr. B. J. Kosakowski
Mr. K. R. Newman
Mr. M. Davis

RW. McCuen

PM HAZARDOUS WASTE FORM

Item	Chemical Name of Waste*	Amount Discarded (weight and/or volume)	Generator and Date
liquid	benzo(a)pyrene	3.5 µg/ml; 10ml	R. McCuen; 8/4/80
"	2-acetylaminofluorence	50 µg/ml; 10ml	" "
"	ethyl methanesulfonate	686 µg/ml; 10ml	" "
"	" "	100 µg	" "
"	dimethyl sulfoxide	50 ml	" 8/8/80
"	nicotine	30 mg/ml; 200ml	" 8/10/80

* no abbreviations; list complete name of all chemicals

GENERATED BY
BUILDING/ROOM NUMBER - Tower/520
RESPONSIBLE PERSON - project leader's signature
DATE - that the project leader signed the form

I certify that the above named materials are properly classified and described according to applicable regulations of the DOT.

manager of the division
name and title - typed

Date

Signature

1003118047

APPENDIX C

*TY 1-200

1.000	011111	69.6	81.7	100.0	44.9
2.000	011112	35.7	75.0	47.0	168.9
3.000	021111	78.8	92.3	100.0	41.4
4.000	021112	55.1	66.7	50.5	151.0
5.000	021112	57.1	78.0	61.3	148.7
6.000	031111	75.8	96.7	100.0	29.3
7.000	031112	54.6	90.0	67.0	116.7
8.000	041111	88.5	115.0	100.0	23.2
9.000	041112	69.8	97.3	66.7	127.7
10.000	052111	80.1	73.3	100.0	50.0
11.000	052112	62.4	73.2	62.0	159.1
12.000	062111	66.4	93.3	100.0	25.0
13.000	062112	38.6	56.7	35.2	244.1
14.000	072111	72.2	70.0	100.0	42.9
15.000	072112	52.9	68.3	72.0	153.7
16.000	072211	80.2	83.3	100.0	24.4
17.000	072212	66.8	63.3	101.0	80.0
18.000	082211	61.4	78.3	100.0	46.4
19.000	082212	51.4	85.7	91.6	128.4
20.000	092211	64.3	96.7	100.0	28.9
21.000	092212	51.7	84.0	69.9	114.3
22.000	102211	57.9	78.0	100.0	27.4
23.000	102212	48.7	63.3	68.3	135.8
24.000	102311	60.7	81.7	100.0	31.8
25.000	102312	43.8	76.7	67.8	112.6
26.000	112311	69.4	93.7	100.0	20.6
27.000	112312	50.1	56.3	43.0	182.2
28.000	122311	36.1	85.7	100.0	34.6
29.000	122312	24.3	67.7	53.0	142.9
30.000	132311	60.3	83.3	100.0	21.2
31.000	132312	38.4	74.3	56.8	122.9
32.000	142311	64.3	84.3	100.0	35.2
33.000	142311	63.1	81.0	100.0	48.6
34.000	142312	50.1	62.0	56.3	161.2
35.000	152311	54.7	75.0	100.0	23.9
36.000	152312	43.5	69.3	73.5	122.9
37.000	162311	55.6	63.0	100.0	22.8
38.000	162312	39.1	73.3	82.0	79.5
39.000	173311	81.3	88.0	100.0	25.6
40.000	173312	53.6	70.0	52.4	164.4
41.000	173314	1.7	55.7	1.3	213.5
42.000	183311	76.6	105.0	100.0	22.2
43.000	183312	50.8	81.7	52.0	129.4
44.000	183314	6.8	101.7	9.0	58.4
45.000	193311	68.0	90.0	100.0	25.5
46.000	193312	58.7	85.3	81.8	107.3
47.000	193314	5.0	78.3	6.4	72.3
48.000	203311	59.8	95.0	100.0	28.5
49.000	203312	53.1	72.7	67.9	157.5
50.000	203314	1.6	64.3	1.8	141.0
51.000	213311	94.4	80.0	100.0	26.0
52.000	213312	62.8	83.3	69.3	135.5
53.000	213312	62.1	51.3	42.2	221.1
54.000	213313	20.8	47.7	13.1	169.3
55.000	213313	24.1	58.3	18.6	103.6
56.000	223311	83.3	93.1	100.0	20.7
57.000	223312	42.3	71.3	38.3	146.3
58.000	223313	13.0	64.7	10.8	150.5
59.000	233311	79.3	79.3	100.0	33.7
60.000	233312	60.3	91.0	87.2	96.3
61.000	233313	16.0	72.0	18.3	135.3
62.000	243311	90.4	83.3	100.0	28.0

44.9
41.4

37.998

10031180 49

64.000	243313	36.7	55.3	24.4	92.5
65.000	253311	86.3	63.7	100.0	34.6
66.000	253312	58.1	55.7	59.5	142.1
67.000	253312	55.3	59.7	60.1	143.3
68.000	253313	24.4	66.3	29.5	110.0
69.000	264311	93.5	82.0	100.0	15.2
70.000	264312	66.2	56.0	48.4	204.5
71.000	264313	19.2	66.3	16.6	102.5
72.000	264314	1.1	36.7	0.5	181.8
73.000	274311	81.5	55.0	100.0	35.6
74.000	274312	54.8	55.3	67.6	172.2
75.000	274313	20.2	36.0	16.0	209.3
76.000	274314	1.2	44.3	1.2	159.4
77.000	284311	77.5	76.7	100.0	31.0
78.000	284312	57.7	73.3	70.8	182.4
79.000	284313	27.9	51.7	24.3	161.3
80.000	284314	0.9	78.0	1.0	180.0
81.000	294311	89.7	80.0	100.0	25.4
82.000	294312	57.8	86.7	70.4	143.7
83.000	294313	27.4	54.7	21.0	136.0
84.000	294314	3.4	67.7	3.0	130.0
85.000	304311	110.6	90.0	100.0	16.6
86.000	304312	77.0	82.3	70.0	124.2
87.000	304313	47.9	82.0	43.8	59.9
88.000	304411	99.6	52.3	100.0	30.9
89.000	304412	77.2	62.0	91.8	124.1
90.000	304413	28.7	56.7	31.1	136.7
91.000	315311	108.8	89.3	100.0	13.7
92.000	315312	83.0	65.0	55.4	184.0
93.000	315313	35.4	53.3	19.5	160.9
94.000	315314	4.3	67.0	2.9	157.4
95.000	315321	108.8	83.7	100.0	23.4
96.000	315322	83.0	68.3	62.2	180.5
97.000	315323	35.4	51.7	20.1	161.3
98.000	315324	4.3	59.0	2.8	172.6
99.000	325321	120.9	87.0	100.0	26.8
100.000	325322	93.7	62.7	55.8	186.7
101.000	325323	44.4	64.3	27.1	154.5
102.000	325324	3.1	28.3	0.8	385.3
103.000	335321	80.6	76.3	100.0	26.2
104.000	335322	80.3	60.0	78.6	202.8
105.000	335323	38.5	60.0	37.6	107.2
106.000	345321	104.4	48.7	100.0	62.3
107.000	345322	75.9	53.7	80.1	224.0
108.000	345323	41.7	51.3	42.1	171.5
109.000	345324	17.1	79.0	26.2	114.7
110.000	355321	110.7	81.0	100.0	34.5
111.000	355322	84.4	46.7	43.9	220.5
112.000	355323	46.9	59.3	30.9	185.7
113.000	365321	66.4	56.0	100.0	44.0
114.000	365322	54.1	56.7	82.3	241.1
115.000	365323	29.0	56.3	43.9	135.6
116.000	375321	78.4	73.3	100.0	34.1
117.000	375322	65.4	60.0	68.2	172.0
118.000	375323	39.8	55.0	38.0	139.2
119.000	385421	70.3	75.0	100.0	46.7
120.000	385422	16.7	40.7	12.9	274.5
121.000	385423	24.4	48.0	21.9	241.7
122.000	395421	44.8	65.0	100.0	67.3
123.000	395422	12.3	65.3	27.7	144.6
124.000	395423	13.4	49.3	22.7	189.9
125.000	405421	100.0	60.7	100.0	46.6
126.000	405422	77.9	51.0	65.4	149.2

127.000	405423	31.3	63.0	32.4	103.3
128.000	415421	101.0	90.7	100.0	19.3
129.000	415422	64.6	56.3	39.6	181.5
130.000	415423	33.8	53.7	19.8	156.6
131.000	425421	79.8	62.0	100.0	40.9
132.000	425422	52.0	71.3	74.8	168.4
133.000	425423	18.4	63.3	23.5	121.7
134.000	425423	16.2	60.3	19.7	116.6
135.000	435421	80.8	94.0	100.0	24.3
136.000	435422	63.4	83.7	69.8	117.9
137.000	435423	39.8	75.3	39.5	132.0
138.000	435424	27.0	55.7	19.8	93.3
139.000	445421	99.8	102.3	100.0	17.3
140.000	445422	76.8	73.3	55.1	131.1
141.000	445423	43.1	85.3	36.0	105.4
142.000	445424	27.1	75.5	16.4	91.3
143.000	455421	93.3	84.0	100.0	42.6
144.000	455422	75.5	71.7	68.9	126.7
145.000	455423	37.0	64.7	30.4	92.3
146.000	465421	94.0	63.0	100.0	33.1
147.000	465422	41.6	71.0	49.8	148.2
148.000	465423	32.8	54.7	30.3	151.9
149.000	475421	81.2	57.3	100.0	19.6
150.000	475422	62.2	47.3	63.3	149.1
151.000	475423	41.3	42.3	37.6	141.2
152.000	475424	16.4	38.7	13.6	98.8
153.000	485421	65.1	69.7	100.0	32.2
154.000	485422	52.3	39.7	45.7	165.3
155.000	485423	5.9	30.7	4.0	319.1
156.000	485424	24.5	64.7	35.0	114.8
157.000	485424	21.9	60.0	29.0	121.5
158.000	485424	20.2	49.7	22.2	136.1
159.000	496421	99.2	57.7	100.0	60.6
160.000	496422	68.6	52.3	62.8	221.4
161.000	496423	14.6	46.7	11.9	217.0
162.000	506421	88.3	60.0	100.0	52.4
163.000	506422	64.7	50.3	62.0	235.5
164.000	506423	14.4	30.7	18.1	360.8

--EOF HIT AFTER 164.
*END

!

APPENDIX D

RUN

ANALYSES FOR SUSPGR

ERROR WITHIN EXPERIMENTS

21112	2.00
142311	.72
213312	.24
213313	5.44
253312	3.92
425423	2.42
485424	4.69

POOLED ERROR VARIANCE= 3.02 8.D.F.

ANOVA WITHIN CELL-MICROS-SEL.AGNT. CLASS

ANOVA FOR CLASS 111

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
1	69.60	35.70	.00	.00	105.30
2	78.80	57.10	.00	.00	135.90
3	75.80	54.60	.00	.00	130.40
4	88.50	69.80	.00	.00	158.30
	4.00	4.00	.00	.00	
	78.17	54.30	.00	.00	
	62.06	198.05	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	1140.0313	1.0000	1140.0313	49.1520	.0049
EXPERS	710.7344	3.0000	236.9115	10.2143	.0479
RESIDL	69.5820	3.0000	23.1940	.0000	.0000

R & D

100311853

TOTAL	1920.3477	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 211

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
5	80.10	62.40	.00	.00	142.50
6	66.40	38.60	.00	.00	105.00
7	72.20	52.90	.00	.00	125.10
	3.00	3.00	.00	.00	
	72.90	51.30	.00	.00	
	47.29	143.53	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	699.8516	1.0000	699.8516	47.5294	.0168
EXPERS	352.1875	2.0000	176.0938	11.9591	.0729
RESIDL	29.4492	2.0000	14.7246	.0000	.0000
TOTAL	1081.4883	5.0000	.0000	.0000	.0000

ANOVA FOR CLASS 221

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
7	80.20	66.80	.00	.00	147.00
8	61.40	51.40	.00	.00	112.80
9	64.30	51.70	.00	.00	116.00
10	57.90	48.70	.00	.00	106.60
	4.00	4.00	.00	.00	
	65.95	54.65	.00	.00	
	97.10	67.43	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	255.3906	1.0000	255.3906	126.1351	.0012
EXPERS	487.5234	3.0000	162.5078	80.2611	.0024

R & D

RESIDL	6.0742	3.0000	2.0247	.0000	.0000
TOTAL	748.9883	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 231

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
10	60.70	43.80	.00	.00	104.50
11	69.40	50.10	.00	.00	119.50
12	36.10	24.30	.00	.00	60.40
13	60.30	38.40	.00	.00	98.70
14	63.10	50.10	.00	.00	113.20
15	54.70	43.50	.00	.00	98.20
16	55.60	39.10	.00	.00	94.70
	7.00	7.00	.00	.00	
	57.13	41.33	.00	.00	
	109.92	78.04	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	873.7344	1.0000	873.7344	109.0570	.0002
EXPERS	1079.6797	6.0000	179.9466	22.4604	.0037
RESIDL	48.0703	6.0000	8.0117	.0000	.0000
TOTAL	2001.4844	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 331

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
17	81.30	53.60	.00	1.70	134.90
18	76.60	50.80	.00	6.80	127.40
19	68.00	58.70	.00	5.00	126.70
20	59.80	53.10	.00	1.60	112.90
21	94.40	62.10	24.10	.00	156.50
22	83.30	42.30	13.00	.00	125.60
23	79.30	60.30	16.00	.00	139.60
24	90.40	62.30	36.70	.00	152.70
25	86.30	55.30	24.40	.00	141.60

	9.00	9.00	5.00	4.00
	79.93	55.39	22.84	3.77
	116.49	41.10	84.93	6.56

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	2710.9375	1.0000	2710.9375	44.3337	.0003
EXPERS	771.5625	8.0000	96.4453	1.5772	.2437
RESIDL	489.1875	8.0000	61.1484	.0000	.0000
TOTAL	3971.6875	17.0000	.0000	.0000	.0000

ANOVA FOR CLASS 431

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
26	93.50	66.20	19.20	1.10	159.70
27	81.50	54.80	20.20	1.20	136.30
28	77.50	57.70	27.90	.90	135.20
29	89.70	57.80	27.40	3.40	147.50
30	110.60	77.00	47.90	.00	187.60

	5.00	5.00	5.00	4.00
	90.56	62.70	28.52	1.65
	165.92	82.04	133.33	1.38

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	1940.4375	1.0000	1940.4375	133.9315	.0011
EXPERS	933.8750	4.0000	233.4688	16.1143	.0170
RESIDL	57.9531	4.0000	14.4883	.0000	.0000
TOTAL	2932.2656	9.0000	.0000	.0000	.0000

ANOVA FOR CLASS 441

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
30	99.60	77.20	28.70	.00	176.80

R & D

100311805G

	1.00	1.00	1.00	.00
	99.60	77.20	28.70	.00
	.00	.00	.00	.00

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	250.8789	1.0000	250.8789	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	250.8789	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 531

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
31	108.80	83.00	35.40	4.30	191.80
	1.00	1.00	1.00	1.00	
	108.80	83.00	35.40	4.30	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	332.8203	1.0000	332.8203	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	332.8203	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 532

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
31	108.80	83.00	35.40	4.30	191.80
32	120.90	93.70	44.40	3.10	214.60
33	80.60	80.30	38.50	.00	160.90
34	104.40	75.90	41.70	17.10	180.30
35	110.70	84.40	46.90	.00	195.10
36	66.40	54.10	29.00	.00	120.50

37	78.40	65.40	39.80	.00	143.80
	7.00	7.00	7.00	3.00	
	95.74	76.69	39.39	8.17	
	415.53	173.35	35.28	60.21	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	1271.1875	1.0000	1271.1875	22.1920	.0038
EXPERS	3189.5625	6.0000	531.5938	9.2804	.0223
RESIDL	343.6875	6.0000	57.2813	.0000	.0000
TOTAL	4804.4375	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 542

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
38	70.30	16.70	24.40	.00	87.00
39	44.80	12.30	13.40	.00	57.10
40	100.00	77.90	31.30	.00	177.90
41	101.00	64.60	33.80	.00	165.60
42	79.80	52.00	16.20	.00	131.80
43	80.80	63.40	39.80	27.00	144.20
44	99.80	76.80	43.10	27.10	176.60
45	93.30	75.50	37.00	.00	168.80
46	94.00	41.60	32.80	.00	135.60
47	81.20	62.20	41.30	16.40	143.40
48	65.10	52.30	5.90	20.20	117.40
	11.00	11.00	11.00	4.00	
	82.74	54.12	29.00	22.67	
	308.09	511.20	153.55	27.93	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	4504.5000	1.0000	4504.5000	46.7818	.0001
EXPERS	7230.0625	10.0000	723.0061	7.5088	.0200
RESIDL	962.8750	10.0000	96.2875	.0000	.0000

R & D

TOTAL	12697.4375	21.0000	.0000	.0000	.0000

ANOVA FOR CLASS 642

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
49	99.20	68.60	14.60	.00	167.80
50	88.30	61.70	14.40	.00	153.00
	2.00	2.00	2.00	.00	
	93.75	66.65	14.50	.00	
	59.40	7.60	.02	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	734.4453	1.0000	734.4453	60.1850	.0835
EXPERS	54.7930	1.0000	54.7930	4.4901	.2876
RESIDL	12.2031	1.0000	12.2031	.0000	.0000
TOTAL	801.4414	3.0000	.0000	.0000	.0000.

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR DMSO

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	8573.4375	10.0000	857.3438	4.3577	.0005
RETEXP	8460.0000	43.0000	196.7142	.0000	.0000
TOTAL	17033.4375	53.0000	321.3855	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
531	1.	108.80
441	1.	99.60
532	7.	95.74
642	2.	93.75
431	5.	90.56
542	11.	82.74
331	9.	79.93
111	4.	78.17
211	3.	72.90

R & D

221	4.	65.95
231	7.	57.13

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR BAP

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	6100.6875	10.0000	610.0686	3.1351	.0046
BETEXP	8367.6250	43.0000	194.5959	.0000	.0000
TOTAL	14468.3125	53.0000	272.9868	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
531	1.	83.00
441	1.	77.20
532	7.	76.69
642	2.	66.65
431	5.	62.70
331	9.	55.39
221	4.	54.65
111	4.	54.30
542	11.	54.12
211	3.	51.30
231	7.	41.33

ANALYSES FOR %CLNEF

ERROR WITHIN EXPERIMENTS

21112	63.84
142311	5.44
213312	511.99
213313	56.18
253312	8.00
425423	4.50
485424	58.86

POOLED ERROR VARIANCE= 95.96 8.D.F.

R & D

ANOVA WITHIN CELL-MICROS-SEL.AGNT. CLASS

ANOVA FOR CLASS 111

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
1	81.70	75.00	.00	.00	156.70
2	92.30	78.00	.00	.00	170.30
3	96.70	90.00	.00	.00	186.70
4	115.00	97.30	.00	.00	212.30
	4.00	4.00	.00	.00	
	96.42	85.07	.00	.00	
	192.98	108.43	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	257.6250	1.0000	257.6250	16.7561	.0245
EXPERS	858.1250	3.0000	286.0415	18.6043	.0211
RESIDL	46.1250	3.0000	15.3750	.0000	.0000
TOTAL	1161.8750	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 211

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
5	73.30	73.20	.00	.00	146.50
6	93.30	56.70	.00	.00	150.00
7	70.00	68.30	.00	.00	138.30
	3.00	3.00	.00	.00	
	78.87	66.07	.00	.00	
	158.96	71.80	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	245.7813	1.0000	245.7813	1.1554	.3959
EXPERS	36.1016	2.0000	18.0508	.0849	.7904
RESIDL	425.4336	2.0000	212.7168	.0000	.0000
TOTAL	707.3164	5.0000	.0000	.0000	.0000

ANOVA FOR CLASS 221

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
7	83.30	63.30	.00	.00	146.60
8	78.30	85.70	.00	.00	164.00
9	96.70	84.00	.00	.00	180.70
10	78.00	63.30	.00	.00	141.30
	4.00	4.00	.00	.00	
	84.07	74.07	.00	.00	
	76.76	155.29	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	199.9844	1.0000	199.9844	2.7766	.1939
EXPERS	480.0820	3.0000	160.0273	2.2218	.2327
RESIDL	216.0742	3.0000	72.0247	.0000	.0000
TOTAL	896.1406	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 231

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
10	81.70	76.70	.00	.00	158.40
11	93.70	56.30	.00	.00	150.00
12	85.70	67.70	.00	.00	153.40
13	83.30	74.30	.00	.00	157.60
14	81.00	62.00	.00	.00	143.00
15	75.00	69.30	.00	.00	144.30
16	63.00	73.30	.00	.00	136.30
	7.00	7.00	.00	.00	
	80.49	68.51	.00	.00	

R & D

	91.23	52.72	.00	.00

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	501.6250	1.0000	501.6250	4.5426	.0755
EXPERS	201.1250	6.0000	33.5208	.3036	.6054
RESIDL	662.5625	6.0000	110.4271	.0000	.0000
TOTAL	1365.3125	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 331

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
17	88.00	70.00	.00	55.70	158.00
18	105.00	81.70	.00	101.70	186.70
19	90.00	85.30	.00	78.30	175.30
20	95.00	72.70	.00	64.30	167.70
21	80.00	51.30	58.30	.00	131.30
22	93.10	71.30	64.70	.00	164.40
23	79.30	91.00	72.00	.00	170.30
24	83.30	74.00	55.30	.00	157.30
25	63.70	59.70	66.30	.00	123.40
	9.00	9.00	5.00	4.00	
	86.38	73.00	63.32	75.00	
	137.08	151.47	43.92	403.58	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	805.3750	1.0000	805.3750	9.8067	.0137
EXPERS	1651.4375	8.0000	206.4297	2.5136	.1493
RESIDL	657.0000	8.0000	82.1250	.0000	.0000
TOTAL	3113.8125	17.0000	.0000	.0000	.0000

ANOVA FOR CLASS 431

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
26	82.00	56.00	66.30	36.70	138.00

27	55.00	55.30	36.00	44.30	110.30
28	76.70	73.30	51.70	78.00	150.00
29	80.00	86.70	54.70	67.70	166.70
30	90.00	82.30	82.00	.00	172.30
	5.00	5.00	5.00	4.00	
	76.74	70.72	58.14	56.67	
	171.69	212.66	294.84	376.15	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	90.5859	1.0000	90.5859	1.1868	.3383
EXPERS	1232.0664	4.0000	308.0166	4.0353	.1144
RESIDL	305.3242	4.0000	76.3311	.0000	.0000
TOTAL	1627.9766	9.0000	.0000	.0000	.0000

ANOVA FOR CLASS 441

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
30	52.30	62.00	56.70	.00	114.30
	1.00	1.00	1.00	.00	
	52.30	62.00	56.70	.00	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	47.0469	1.0000	47.0469	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	47.0469	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 531

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
31	89.30	65.00	53.30	67.00	154.30

R & D

	1.00	1.00	1.00	1.00
	89.30	65.00	53.30	67.00
	.00	.00	.00	.00

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	295.2461	1.0000	295.2461	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	295.2461	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 532

EXPER	£ DMSO	BAP	2AAP	2R1200	DMSO+BAP
31	83.70	68.30	51.70	59.00	152.00
32	87.00	62.70	64.30	28.30	149.70
33	76.30	60.00	60.00	.00	136.30
34	48.70	53.70	51.30	79.00	102.40
35	81.00	46.70	59.30	.00	127.70
36	56.00	56.70	56.30	.00	112.70
37	73.30	60.00	55.00	.00	133.30
	7.00	7.00	7.00	3.00	
	72.29	58.30	56.84	55.43	
	210.24	47.24	22.08	652.16	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	684.5938	1.0000	684.5938	7.4481	.0335
EXPERS	993.3555	6.0000	165.5592	1.8012	.2271
RESIDL	551.4922	6.0000	91.9154	.0000	.0000
TOTAL	2229.4414	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 542

EXPER	£ DMSO	BAP	2AAP	2R1200	DMSO+BAP

38	75.00	40.70	48.00	.00	115.70
39	65.00	65.30	49.30	.00	130.30
40	60.70	51.00	63.00	.00	111.70
41	90.70	56.30	53.70	.00	147.00
42	62.00	71.30	60.30	.00	133.30
43	94.00	83.70	75.30	55.70	177.70
44	102.30	73.30	85.30	75.50	175.60
45	84.00	71.70	64.70	.00	155.70
46	63.00	71.00	54.70	.00	134.00
47	57.30	47.30	42.30	38.70	104.60
48	69.70	39.70	30.70	49.70	109.40
	11.00	11.00	11.00	4.00	
	74.88	61.03	57.03	54.90	
	239.26	218.13	230.63	238.16	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	1055.6875	1.0000	1055.6875	8.1465	.0165
EXPERS	3278.0000	10.0000	327.7998	2.5296	.1403
RESIDL	1295.8750	10.0000	129.5875	.0000	.0000
TOTAL	5629.5625	21.0000	.0000	.0000	.0000

ANOVA FOR CLASS 642

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
49	57.70	52.30	46.70	.00	110.00
50	60.00	50.30	30.70	.00	110.30
	2.00	2.00	2.00	.00	
	58.85	51.30	38.70	.00	
	2.64	2.00	128.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	57.0039	1.0000	57.0039	12.3356	.1852

R & D

EXPERS	.0234	1.0000	.0234	.0051	.9557
RESIDL	4.6211	1.0000	4.6211	.0000	.0000
TOTAL	61.6484	3.0000	.0000	.0000	.0000

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR DMSO

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	3981.3750	10.0000	398.1375	2.4064	.0224
BETEXP	7114.2500	43.0000	165.4477	.0000	.0000
TOTAL	11095.6250	53.0000	209.3514	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
111	4.	96.42
531	1.	89.30
331	9.	86.38
221	4.	84.07
231	7.	80.49
211	3.	78.87
431	5.	76.74
542	11.	74.88
532	7.	72.29
642	2.	58.85
441	1.	52.30

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR BAP

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	3358.5625	10.0000	335.8562	2.4987	.0182
BETEXP	5779.7500	43.0000	134.4128	.0000	.0000
TOTAL	9138.3125	53.0000	172.4210	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
111	4.	85.07
221	4.	74.07
331	9.	73.00
431	5.	70.72

R & D

231	7.	68.51
211	3.	66.07
531	1.	65.00
441	1.	62.00
542	11.	61.03
532	7.	58.30
642	2.	51.30

ANALYSES FOR %SURVI

ERROR WITHIN EXPERIMENTS

21112	58.32
142311	.00
213312	367.20
213313	15.13
253312	.18
425423	7.22
485424	41.01

POOLED ERROR VARIANCE= 66.26 8.D.F.

ANOVA WITHIN CELL-MICROS-SEL.AGNT. CLASS

ANOVA FOR CLASS 111

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
1	100.00	47.00	.00	.00	147.00
2	100.00	61.30	.00	.00	161.30
3	100.00	67.00	.00	.00	167.00
4	100.00	66.70	.00	.00	166.70
	4.00	4.00	.00	.00	
	100.00	60.50	.00	.00	
	.00	87.86	.00	.00	

R & D

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	3120.5000	1.0000	3120.5000	71.0340	.0028
EXPERS	131.7852	3.0000	43.9284	1.0000	.6073
RESIDL	131.7891	3.0000	43.9297	.0000	.0000
TOTAL	3384.0742	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 211

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
5	100.00	62.00	.00	.00	162.00
6	100.00	35.20	.00	.00	135.20
7	100.00	72.00	.00	.00	172.00
	3.00	3.00	.00	.00	
	100.00	56.40	.00	.00	
	.00	362.08	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	2851.4492	1.0000	2851.4492	15.7508	.0557
EXPERS	362.0938	2.0000	181.0469	1.0001	.4238
RESIDL	362.0703	2.0000	181.0352	.0000	.0000
TOTAL	3575.6133	5.0000	.0000	.0000	.0000

ANOVA FOR CLASS 221

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
7	100.00	101.00	.00	.00	201.00
8	100.00	91.60	.00	.00	191.60
9	100.00	69.90	.00	.00	169.90
10	100.00	68.30	.00	.00	168.30
	4.00	4.00	.00	.00	
	100.00	82.70	.00	.00	

.00	261.78	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	598.6250	1.0000	598.6250	4.5740	.1214
EXPERS	392.6875	3.0000	130.8958	1.0002	.3927
RESIDL	392.6250	3.0000	130.8750	.0000	.0000
TOTAL	1383.9375	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 231

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
10	100.00	67.80	.00	.00	167.80
11	100.00	43.00	.00	.00	143.00
12	100.00	53.00	.00	.00	153.00
13	100.00	56.80	.00	.00	156.80
14	100.00	56.30	.00	.00	156.30
15	100.00	73.50	.00	.00	173.50
16	100.00	82.00	.00	.00	182.00

7.00	7.00	.00	.00
100.00	61.77	.00	.00
.00	177.84	.00	.00

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	5115.0625	1.0000	5115.0625	57.5332	.0006
EXPERS	533.6250	6.0000	88.9375	1.0004	.3578
RESIDL	533.4375	6.0000	88.9063	.0000	.0000
TOTAL	6182.1250	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 331

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
17	100.00	52.40	.00	1.30	152.40
18	100.00	52.00	.00	9.00	152.00
19	100.00	81.80	.00	6.40	181.80
20	100.00	67.90	.00	1.80	167.90

21	100.00	42.20	18.60	.00	142.20
22	100.00	38.30	10.80	.00	138.30
23	100.00	87.20	18.30	.00	187.20
24	100.00	61.20	24.40	.00	161.20
25	100.00	60.10	29.50	.00	160.10
	9.00	9.00	5.00	4.00	
	100.00	60.34	20.32	4.62	
	.00	273.43	49.65	13.78	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	7076.5000	1.0000	7076.5000	51.7595	.0002
EXPERS	1093.7500	8.0000	136.7188	1.0000	.3485
RESIDL	1093.7500	8.0000	136.7188	.0000	.0000
TOTAL	9264.0000	17.0000	.0000	.0000	.0000

ANOVA FOR CLASS 431

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
26	100.00	48.40	16.60	.50	148.40
27	100.00	67.60	16.00	1.20	167.60
28	100.00	70.80	24.30	1.00	170.80
29	100.00	70.40	21.00	3.00	170.40
30	100.00	70.00	43.80	.00	170.00
	5.00	5.00	5.00	4.00	
	100.00	65.44	24.34	1.42	
	.00	92.29	129.83	1.19	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	2986.0000	1.0000	2986.0000	64.7152	.0024
EXPERS	184.5625	4.0000	46.1406	1.0000	.3758
RESIDL	184.5625	4.0000	46.1406	.0000	.0000
TOTAL	3355.1250	9.0000	.0000	.0000	.0000

ANOVA FOR CLASS 441

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
30	100.00	91.80	31.10	.00	191.80
	1.00	1.00	1.00	.00	
	100.00	91.80	31.10	.00	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	33.6211	1.0000	33.6211	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	33.6211	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 531

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
31	100.00	55.40	19.50	2.90	155.40
	1.00	1.00	1.00	1.00	
	100.00	55.40	19.50	2.90	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	994.5781	1.0000	994.5781	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	994.5781	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 532

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP

31	100.00	62.20	20.10	2.80	162.20
32	100.00	55.80	27.10	.80	155.80
33	100.00	78.60	37.60	.00	178.60
34	100.00	80.10	42.10	26.20	180.10
35	100.00	43.90	30.90	.00	143.90
36	100.00	82.30	43.90	.00	182.30
37	100.00	68.20	38.00	.00	168.20
	7.00	7.00	7.00	3.00	
	100.00	67.30	34.24	9.93	
	.00	203.87	73.77	199.45	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	3742.5625	1.0000	3742.5625	36.7180	.0014
EXPERS	611.6250	6.0000	101.9375	1.0001	.3579
RESIDL	611.5625	6.0000	101.9271	.0000	.0000
TOTAL	4965.7500	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 542

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
38	100.00	12.90	21.90	.00	112.90
39	100.00	27.70	22.70	.00	127.70
40	100.00	65.40	32.40	.00	165.40
41	100.00	39.60	19.80	.00	139.60
42	100.00	74.80	19.70	.00	174.80
43	100.00	69.80	39.50	19.80	169.80
44	100.00	55.10	36.00	16.40	155.10
45	100.00	68.90	30.40	.00	168.90
46	100.00	49.80	30.30	.00	149.80
47	100.00	63.30	37.60	13.60	163.30
48	100.00	45.70	4.00	22.20	145.70
	11.00	11.00	11.00	4.00	
	100.00	52.09	26.75	18.00	
	.00	375.67	107.92	14.27	

R & D

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	12624.1250	1.0000	12624.1250	67.2077	.0001
EXPERS	1878.3125	10.0000	187.8312	1.0000	.6576
RESIDL	1878.3750	10.0000	187.8375	.0000	.0000
TOTAL	16380.8125	21.0000	.0000	.0000	.0000

ANOVA FOR CLASS 642

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
49	100.00	62.80	11.90	.00	162.80
50	100.00	62.00	18.10	.00	162.00
	2.00	2.00	2.00	.00	
	100.00	62.40	15.00	.00	
	.00	.32	19.22	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	1413.8008	1.0000	1413.8008	11675.2578	.0094
EXPERS	.1992	1.0000	.1992	1.6452	.4238
RESIDL	.1211	1.0000	.1211	.0000	.0000
TOTAL	1414.1211	3.0000	.0000	.0000	.0000

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR DMSO

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	.0000	10.0000	.0000	.0000	1.0000
BETEXP	.0000	43.0000	.0000	.0000	.0000
TOTAL	.0000	53.0000	.0000	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
642	2.	100.00
542	11.	100.00
532	7.	100.00

R & D

531	1.	100.00
441	1.	100.00
431	5.	100.00
331	9.	100.00
231	7.	100.00
221	4.	100.00
211	3.	100.00
111	4.	100.00

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR BAP

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	4107.0625	10.0000	410.7061	1.7020	.1113
BETEXP	10376.2500	43.0000	241.3081	.0000	.0000
TOTAL	14483.3125	53.0000	273.2700	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
441	1.	91.80
221	4.	82.70
532	7.	67.30
431	5.	65.44
642	2.	62.40
231	7.	61.77
111	4.	60.50
331	9.	60.34
211	3.	56.40
531	1.	55.40
542	11.	52.09

ANALYSES FOR MUTFRQ

ERROR WITHIN EXPERIMENTS

21112	2.69
142311	89.78
213312	3663.72

213313 2158.28
253312 .75
425423 13.00
485424 118.67
POOLED ERROR VARIANCE=770.69 8.D.F.

ANOVA WITHIN CELL-MICROS-SEL.AGNT. CLASS

ANOVA FOR CLASS 111

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
1	44.90	168.90	.00	.00	213.80
2	41.40	148.70	.00	.00	190.10
3	29.30	116.70	.00	.00	146.00
4	23.20	127.70	.00	.00	150.90
	4.00	4.00	.00	.00	
	34.70	140.50	.00	.00	
	103.45	534.71	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	22387.2500	1.0000	22387.2500	199.3624	.0007
EXPERS	1577.6172	3.0000	525.8723	4.6830	.1185
RESIDL	336.8828	3.0000	112.2943	.0000	.0000
TOTAL	24301.7500	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 211

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
5	50.00	159.10	.00	.00	209.10
6	25.00	244.10	.00	.00	269.10
7	42.90	153.70	.00	.00	196.60
	3.00	3.00	.00	.00	

R & D

39.30	185.63	.00	.00	
165.97	2571.04	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	32120.1875	1.0000	32120.1875	16.1736	.0542
EXPERS	1502.0625	2.0000	751.0313	.3782	.6006
RESIDL	3971.9375	2.0000	1985.9688	.0000	.0000
TOTAL	37594.1875	5.0000	.0000	.0000	.0000

ANOVA FOR CLASS 221

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
7	24.40	80.00	.00	.00	104.40
8	46.40	128.40	.00	.00	174.80
9	28.90	114.30	.00	.00	143.20
10	27.40	135.80	.00	.00	163.20
	4.00	4.00	.00	.00	
	31.77	114.62	.00	.00	
	98.56	612.39	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	13728.2344	1.0000	13728.2344	58.7255	.0038
EXPERS	1431.5664	3.0000	477.1887	2.0413	.2483
RESIDL	701.3086	3.0000	233.7695	.0000	.0000
TOTAL	15861.1094	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 231

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
10	31.80	112.60	.00	.00	144.40
11	20.60	182.20	.00	.00	202.80
12	34.60	142.90	.00	.00	177.50
13	21.20	122.90	.00	.00	144.10
14	48.60	161.20	.00	.00	209.80

R & D

15	23.90	122.90	.00	.00	146.80
16	22.80	79.50	.00	.00	102.30
	7.00	7.00	.00	.00	
	29.07	132.03	.00	.00	
	103.20	1131.63	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	37100.5625	1.0000	37100.5625	71.7092	.0004
EXPERS	4304.6875	6.0000	717.4478	1.3867	.2836
RESIDL	3104.2500	6.0000	517.3750	.0000	.0000
TOTAL	44509.5000	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 331

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
17	25.60	164.40	.00	213.50	190.00
18	22.20	129.40	.00	58.40	151.60
19	25.50	107.30	.00	72.30	132.80
20	28.50	157.50	.00	141.00	186.00
21	26.00	221.10	103.60	.00	247.10
22	20.70	146.30	150.50	.00	167.00
23	33.70	96.30	135.30	.00	130.00
24	28.00	143.80	92.50	.00	171.80
25	34.60	143.30	110.00	.00	177.90
	9.00	9.00	5.00	4.00	
	27.20	145.49	118.38	121.30	
	21.68	1295.56	569.10	5082.13	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	62965.0000	1.0000	62965.0000	90.4101	.0001
EXPERS	4966.4375	8.0000	620.8047	.8914	.6246
RESIDL	5571.5000	8.0000	696.4375	.0000	.0000
TOTAL	73502.9375	17.0000	.0000	.0000	.0000

R & D

ANOVA FOR CLASS 431

EXPER £	DMSO	BAP	2AAF	2R1200	DMSO+BAP
26	15.20	204.50	102.50	181.80	219.70
27	35.60	172.20	209.30	159.40	207.80
28	31.00	182.40	161.30	180.00	213.40
29	25.40	143.70	136.00	130.00	169.10
30	16.60	124.20	59.90	.00	140.80
	5.00	5.00	5.00	4.00	
	24.76	165.40	133.80	162.80	
	78.71	1008.10	3225.58	581.41	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	49449.0625	1.0000	49449.0625	96.7101	.0015
EXPERS	2301.9375	4.0000	575.4844	1.1255	.3499
RESIDL	2045.2500	4.0000	511.3125	.0000	.0000
TOTAL	53796.2500	9.0000	.0000	.0000	.0000

ANOVA FOR CLASS 441

EXPER £	DMSO	BAP	2AAF	2R1200	DMSO+BAP
30	30.90	124.10	136.70	.00	155.00
	1.00	1.00	1.00	.00	
	30.90	124.10	136.70	.00	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	4343.1172	1.0000	4343.1172	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	4343.1172	1.0000	.0000	.0000	.0000

R & D

ANOVA FOR CLASS 531

EXPER £	DMSO	BAP	2AAF	2R1200	DMSO+BAP
31	13.70	184.00	160.90	157.40	197.70
	1.00	1.00	1.00	1.00	
	13.70	184.00	160.90	157.40	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	14501.0469	1.0000	14501.0469	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	14501.0469	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 532

EXPER £	DMSO	BAP	2AAF	2R1200	DMSO+BAP
31	23.40	180.50	161.30	172.60	203.90
32	26.80	186.70	154.50	385.30	213.50
33	26.20	202.80	107.20	.00	229.00
34	62.30	224.00	171.50	114.70	286.30
35	34.50	220.50	185.70	.00	255.00
36	44.00	241.10	135.60	.00	285.10
37	34.10	172.00	139.20	.00	206.10
	7.00	7.00	7.00	3.00	
	35.90	203.94	150.71	224.20	
	183.49	654.29	672.83	20303.04	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	98834.2500	1.0000	98834.2500	499.2678	.0001
EXPERS	3839.0625	6.0000	639.8438	3.2322	.1206

28.

R & D

RESIDL	1187.7500	6.0000	197.9583	.0000	.0000
TOTAL	103861.0625	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 542

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
38	46.70	274.50	241.70	.00	321.20
39	67.30	144.60	189.90	.00	211.90
40	46.60	149.20	103.30	.00	195.80
41	19.30	181.50	156.60	.00	200.80
42	40.90	168.40	116.60	.00	209.30
43	24.30	117.90	132.00	93.30	142.20
44	17.30	131.10	105.40	91.30	148.40
45	42.60	126.70	92.30	.00	169.30
46	33.10	148.20	151.90	.00	181.30
47	19.60	149.10	141.20	98.80	168.70
48	32.20	165.30	319.10	136.10	197.50
	11.00	11.00	11.00	4.00	
	35.45	159.68	159.09	104.87	
	232.80	1799.85	4675.02	443.41	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	84890.5625	1.0000	84890.5625	95.9385	.0001
EXPERS	11478.0625	10.0000	1147.8062	1.2972	.2812
RESIDL	8848.4375	10.0000	884.8438	.0000	.0000
TOTAL	105217.0625	21.0000	.0000	.0000	.0000

ANOVA FOR CLASS 642

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
49	60.60	221.40	217.00	.00	282.00
50	52.40	235.50	360.80	.00	287.90
	2.00	2.00	2.00	.00	
	56.50	228.45	288.90	.00	

R & D

	33.62	99.44	10339.19	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	29566.8125	1.0000	29566.8125	237.8426	.0402
EXPERS	8.6875	1.0000	8.6875	.0699	.8271
RESIDL	124.3125	1.0000	124.3125	.0000	.0000
TOTAL	29699.8125	3.0000	.0000	.0000	.0000

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR DMSO

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	2479.4336	10.0000	247.9434	1.9357	.0659
BETEXP	5508.0000	43.0000	128.0930	.0000	.0000
TOTAL	7987.4336	53.0000	150.7063	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
642	2.	56.50
211	3.	39.30
532	7.	35.90
542	11.	35.45
111	4.	34.70
221	4.	31.77
441	1.	30.90
231	7.	29.07
331	9.	27.20
431	5.	24.76
531	1.	13.70

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR BAP

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	43956.0000	10.0000	4395.5977	3.6496	.0017
BETEXP	51789.0000	43.0000	1204.3953	.0000	.0000
TOTAL	95745.0000	53.0000	1806.5093	.0000	.0000

R & D

CLASS I.D.	#EXPER.	CLASS AVG.
642	2.	228.45
532	7.	203.94
211	3.	185.63
531	1.	184.00
431	5.	165.40
542	11.	159.68
331	9.	145.49
111	4.	140.50
231	7.	132.03
441	1.	124.10
221	4.	114.62

ANALYSES FOR LOGMF

ERROR WITHIN EXPERIMENTS

21112	.00
142311	.05
213312	.12
213313	.12
253312	.00
425423	.00
485424	.01

POOLED ERROR VARIANCE= .04 8.D.F.

ANOVA WITHIN CELL-MICROS-SEL.AGNT. CLASS

ANOVA FOR CLASS 111

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
1	3.80	5.13	.00	.00	8.93
2	3.72	5.00	.00	.00	8.73
3	3.38	4.76	.00	.00	8.14
4	3.14	4.85	.00	.00	7.99

	4.00	4.00	.00	.00
	3.51	4.94	.00	.00
	.09	.03	.00	.00

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	4.0485	1.0000	4.0485	216.8845	.0006
EXPERS	.3078	3.0000	.1026	5.4970	.1000
RESIDL	.0560	3.0000	.0187	.0000	.0000
TOTAL	4.4123	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 211

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
5	3.91	5.07	.00	.00	8.98
6	3.22	5.50	.00	.00	8.72
7	3.76	5.04	.00	.00	8.79
	3.00	3.00	.00	.00	
	3.63	5.20	.00	.00	
	.13	.07	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	3.7011	1.0000	3.7011	19.5145	.0447
EXPERS	.0187	2.0000	.0093	.0493	.8370
RESIDL	.3793	2.0000	.1897	.0000	.0000
TOTAL	4.0991	5.0000	.0000	.0000	.0000

ANOVA FOR CLASS 221

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
7	3.19	4.38	.00	.00	7.58
8	3.84	4.86	.00	.00	8.69
9	3.36	4.74	.00	.00	8.10

R & D

10	3.31	4.91	.00	.00	8.22
	4.00	4.00	.00	.00	
	3.43	4.72	.00	.00	
	.08	.06	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	3.3553	1.0000	3.3553	107.0381	.0016
EXPERS	.3153	3.0000	.1051	3.3532	.1641
RESIDL	.0940	3.0000	.0313	.0000	.0000
TOTAL	3.7647	7.0000	.0000	.0000	.0000

ANOVA FOR CLASS 231

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
10	3.46	4.72	.00	.00	8.18
11	3.03	5.21	.00	.00	8.23
12	3.54	4.96	.00	.00	8.51
13	3.05	4.81	.00	.00	7.87
14	3.88	5.08	.00	.00	8.97
15	3.17	4.81	.00	.00	7.99
16	3.13	4.38	.00	.00	7.50
	7.00	7.00	.00	.00	
	3.32	4.85	.00	.00	
	.10	.07	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	8.1860	1.0000	8.1860	129.7403	.0002
EXPERS	.6617	6.0000	.1103	1.7480	.2334
RESIDL	.3786	6.0000	.0631	.0000	.0000
TOTAL	9.2263	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 331

EXPER £	DMSO	BAP	2AAF	2R1200	DMSO+BAP
17	3.24	5.10	.00	5.36	8.34
18	3.10	4.86	.00	4.07	7.96
19	3.24	4.68	.00	4.28	7.91
20	3.35	5.06	.00	4.95	8.41
21	3.26	5.40	4.64	.00	8.66
22	3.03	4.99	5.01	.00	8.02
23	3.52	4.57	4.91	.00	8.08
24	3.33	4.97	4.53	.00	8.30
25	3.54	4.96	4.70	.00	8.51
	9.00	9.00	5.00	4.00	
	3.29	4.95	4.76	4.67	
	.03	.06	.04	.36	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	12.4539	1.0000	12.4539	234.2641	.0001
EXPERS	.2737	8.0000	.0342	.6435	.5495
RESIDL	.4253	8.0000	.0532	.0000	.0000
TOTAL	13.1528	17.0000	.0000	.0000	.0000

ANOVA FOR CLASS 431

EXPER £	DMSO	BAP	2AAF	2R1200	DMSO+BAP
26	2.72	5.32	4.63	5.20	8.04
27	3.57	5.15	5.34	5.07	8.72
28	3.43	5.21	5.08	5.19	8.64
29	3.23	4.97	4.91	4.87	8.20
30	2.81	4.82	4.09	.00	7.63
	5.00	5.00	5.00	4.00	
	3.15	5.09	4.81	5.08	
	.14	.04	.23	.02	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB

R & D

AGENTS	9.3959	1.0000	9.3959	116.8664	.0012
EXPERS	.4014	4.0000	.1003	1.2480	.3274
RESIDL	.3216	4.0000	.0804	.0000	.0000
TOTAL	10.1188	9.0000	.0000	.0000	.0000

ANOVA FOR CLASS 441

EXPER #	DMSO	BAP	2AAP	2R1200	DMSO+BAP
30	3.43	4.82	4.92	.00	8.25
	1.00	1.00	1.00	.00	
	3.43	4.82	4.92	.00	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	.9665	1.0000	.9665	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000
TOTAL	.9665	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 531

EXPER #	DMSO	BAP	2AAP	2R1200	DMSO+BAP
31	2.62	5.21	5.08	5.06	7.83
	1.00	1.00	1.00	1.00	
	2.62	5.21	5.08	5.06	
	.00	.00	.00	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	3.3736	1.0000	3.3736	.0000	1.0000
EXPERS	.0000	.0000	.0000	.0000	1.0000
RESIDL	.0000	.0000	.0000	.0000	.0000

TOTAL	3.3736	1.0000	.0000	.0000	.0000

ANOVA FOR CLASS 532

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
31	3.15	5.20	5.08	5.15	8.35
32	3.29	5.23	5.04	5.95	8.52
33	3.27	5.31	4.67	.00	8.58
34	4.13	5.41	5.14	4.74	9.54
35	3.54	5.40	5.22	.00	8.94
36	3.78	5.49	4.91	.00	9.27
37	3.53	5.15	4.94	.00	8.68
	7.00	7.00	7.00	3.00	
	3.53	5.31	5.00	5.28	
	.12	.02	.03	.38	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	11.1328	1.0000	11.1328	293.8774	.0001
EXPERS	.5652	6.0000	.0942	2.4866	.1643
RESIDL	.2273	6.0000	.0379	.0000	.0000
TOTAL	11.9253	13.0000	.0000	.0000	.0000

ANOVA FOR CLASS 542

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
38	3.84	5.61	5.49	.00	9.46
39	4.21	4.97	5.25	.00	9.18
40	3.84	5.01	4.64	.00	8.85
41	2.96	5.20	5.05	.00	8.16
42	3.71	5.13	4.76	.00	8.84
43	3.19	4.77	4.88	4.54	7.96
44	2.85	4.88	4.66	4.51	7.73
45	3.75	4.84	4.53	.00	8.59
46	3.50	5.00	5.02	.00	8.50
47	2.98	5.00	4.95	4.59	7.98
48	3.47	5.11	5.77	4.91	8.58

R & D

	11.00	11.00	11.00	4.00
	3.48	5.05	5.00	4.64
	.19	.05	.14	.03

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	13.4702	1.0000	13.4702	135.6627	.0001
EXPERS	1.4504	10.0000	.1450	1.4608	.2538
RESIDL	.9929	10.0000	.0993	.0000	.0000
TOTAL	15.9136	21.0000	.0000	.0000	.0000

ANOVA FOR CLASS 642

EXPER #	DMSO	BAP	2AAF	2R1200	DMSO+BAP
49	4.10	5.40	5.38	.00	9.50
50	3.96	5.46	5.89	.00	9.42
	2.00	2.00	2.00	.00	
	4.03	5.43	5.63	.00	
	.01	.00	.13	.00	

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
AGENTS	1.9580	1.0000	1.9580	185.9739	.0456
EXPERS	.0019	1.0000	.0019	.1812	.7361
RESIDL	.0105	1.0000	.0105	.0000	.0000
TOTAL	1.9705	3.0000	.0000	.0000	.0000

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR DMSO

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	2.2510	10.0000	.2251	2.0073	.0560
BETEXP	4.8220	43.0000	.1121	.0000	.0000
TOTAL	7.0730	53.0000	.1335	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
642	2.	4.03
211	3.	3.63
532	7.	3.53
111	4.	3.51
542	11.	3.48
441	1.	3.43
221	4.	3.43
231	7.	3.32
331	9.	3.29
431	5.	3.15
531	1.	2.62

ANOVA ACROSS CELL-MICROS-SEL.AGNT.CLASSES FOR BAP

SOURCE	SUMSQR	DGFREE	MEANSQ	FRATIO	PROB
CLASS	1.7517	10.0000	.1752	3.6681	.0016
BETEXP	2.0535	43.0000	.0478	.0000	.0000
TOTAL	3.8052	53.0000	.0718	.0000	.0000

CLASS I.D.	#EXPER.	CLASS AVG.
642	2.	5.43
532	7.	5.31
531	1.	5.21
211	3.	5.20
431	5.	5.09
542	11.	5.05
331	9.	4.95
111	4.	4.94
231	7.	4.85
441	1.	4.82
221	4.	4.72

STOP 0

38

R & D